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REVIEW ARTICLE

Duckweeds as edible vaccines in the animal farming industry

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Abstract
Animal diseases are among the most debilitating issues in the animal farming industry, resulting in decreased productivity and product quality worldwide. An emerging alternative to conventional injectable vaccines is edible vaccines, which promise increased delivery efficiency while maintaining vaccine effectiveness. One of the most promising platforms for edible vaccines is duckweeds, due to their high growth rate, ease of transformation, and excellent nutritional content. This review explores the potential, feasibility, and advantages of using duckweeds as platforms for edible vaccines. Duckweeds have proven to be superb feed sources, as evidenced by numerous improvements in both quantity (e.g., weight gain) and quality (e.g., yolk pigmentation). In terms of heterologous protein production, duckweeds, being plants, are capable of expressing proteins with complex structures and post-translational modifications. Research efforts have focused on the development of duckweed-based edible vaccines, including those against avian influenza, tuberculosis, Newcastle disease, and mastitis, among others. As with any emerging technology, the development of duckweeds as a platform for edible vaccines is still in its early stages compared to well-established injectable vaccines. It is evident that more proof-of-concept studies are required to bring edible vaccines closer to the current standards of conventional vaccines. Specifically, the duckweed expression system needs further development in areas such as yield and growth rate, especially when compared to bacterial and mammalian expression systems. Continued efforts in this field could lead to breakthroughs that significantly improve the resilience of the animal farming industry against disease threats.

Keywords Duckweeds · Edible vaccines · Vaccination · Livestock · Fisheries · Transformation

Introduction

The animal farming industry plays a pivotal role in food production, particularly in meeting global protein demands. Beyond its primary focus on raising chickens, cattle, sheep, goats, birds, and swine (Parisi et al. 2020), the industry also encompasses aquaculture, including fish, shrimp, and shellfish farming (Valenti et al. 2021). While meat and eggs are the predominant products (Henchion et al. 2021), the industry also yields other vital commodities such as milk and leather. Forecasts by Henchion et al. (2021) suggest a continued surge in demand for meat, eggs, and milk until 2050, emphasizing the critical need for sustainable practices within animal farming. This escalating demand for animal protein is driven by the necessity for essential amino acids crucial for human health. Research by Attia et al. (2020) underscores the significance of these amino acids, highlighting their role in supporting various bodily functions. As populations increase and dietary preferences shift, the demand for sustainable animal protein production becomes more pressing (Miassi and Dossa 2023). Consequently, the animal farming industry faces the challenge of meeting increasing demands while minimizing its environmental footprint and ensuring animal welfare. Addressing these complex issues requires interdisciplinary efforts and innovative approaches to ensure the long-term viability of animal farming systems and their contribution to global food security.

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Although animal husbandry has evolved over time, it continues to confront significant challenges. Among these challenges, two major issues stand out prominently: feed management and animal health (Buller et al. 2020). These two factors are crucially important, as they directly impact both the productivity and the welfare of animals within the industry. (Buller et al. 2020). Maintaining animal health is crucial, as farm animals are susceptible to various diseases without proper husbandry practices. Common livestock diseases, as outlined in Table 1, pose substantial threats to animal well-being and productivity. The repercussions of these diseases can manifest through a diverse array of symptoms, which can vary widely in severity from mild to severe. These symptoms may include ruffled feathers, which can indicate discomfort or distress, as well as a noticeable loss of appetite that can lead to further complications. Other potential signs include diarrhoea, weight loss, coughing, paralysis, and depression, reflecting a general decline in well-being, all contribute to the complex and challenging nature of managing these diseases effectively (Serbessa et al. 2023). Traditionally, diseases are often addressed through the administration of drugs and antibiotics, primarily as curative measures rather than preventive ones (Palma et al. 2020). However, preventive measures, such as vaccination, are increasingly recognized as more effective and sustainable approaches to disease management. Preventing diseases through vaccination serves multiple critical purposes. It not only reduces the reliance on antibiotics, which can help decrease the potential for developing antibiotic-resistant strains of bacteria, but also significantly lowers the risk of such resistance becoming a widespread problem (Kumar et al. 2020).

Table 1 Diseases in livestock or fisheries present considerable challenges, affecting productivity and sustainability

Animal	Disease	Caused by	Refs
Chicken	Avian influenza	H5 or H7 virus	Shi et al. (2023)
	Newcastle disease	*Paramyxoviridae* virus	Kgotlele et al. (2020)
	Fowl pox	Fowl poxvirus (FPV)	Fagbohun et al. (2022)
	Salmonellosis	*Salmonella enterica*	Casaux et al. (2023)
	Colibacillosis	Avian pathogenic *Escherichia coli (APEC)*	Apostolakos et al. (2021)
	Mycoplasmosis	*Mycoplasma gallisepticum*	Yadav et al. (2022)
Cattle	Brucellosis	*Brucella abortus*	Blasco et al. (2023)
	Foot and mouth disease	*Picornaviridae* virus	Sun et al. (2020)
	Bluetongue	*Orbivirus* in family *Reoviridae*	Roy (2020)
	Botulism	*Clostridium botulinum*	Rawson et al. (2023)
	Chlamydiosis	*Chlamydia pecorum*	Struthers et al. (2021)
	Leptospirosis	Pathogenic spirochaetes genus *Leptospira*	Samrot et al. (2021)
Goat and sheep	Scabby mouth	*Parapoxvirus*	Bukar et al. (2021)
	Ovine Johne's disease (OJD)	*Mycobacterium avium* subsp. *paratuberculosis* (MAP)	Links et al. (2021)
	Footrot	*Dichelobacter nodosus*	Storms et al. (2021)
	Listeriosis	*Listeria Monocytogenes*	Ravindhiran et al. (2023)
	Barber's pole worm disease	*Haemonchus contortus*	Crilly et al. (2020)
Pig	African swine fever (ASF)	*African swine fever virus* (ASFV)	Salguero (2020)
	Porcine disease	*Porcine parvovirus*	Nelsen et al. (2021)
	Mastitis-Metritis-Agalactia	*E. coli*, *Streptococci* sp., *Staphylococci* sp.	Paramasivam et al. (2023)
	Seborrheic dermatitis (SD)	*Malassezia restricta*	Koga et al. (2020)
	Neonatal coccidiosis	*Cystoisospora suis*	Nunes et al. (2023)
Fish	Columnaris	*Flavobacterium columnare*	LaFrentz et al. (2022)
	Ich	*Ichthyophthirius multifiliis*	Nguyen et al. 2020
	Hemorrhagic disease	Grass carp reovirus	Zhu et al. (2022)
	Velvet	*Piscinoodinium pillulare* and *P. limneticum*	Lieke et al. (2020)
Shrimp	Vibriosis	*Vibrio harveyi*	Abdel-Latif et al. (2022)
	White spot syndrome	*Nimaviridae* (genus *Whispovirus*)	Bao et al. (2020)
Shellfish (mollusks)	Roseovarius oyster disease (ROD)	*Roseovarius crassostreae*	Takyi et al. (2024)
	QPX (Quahog Parasite Unknown) disease	*Quahog Parasite Unknown* (QPX)	Geraci-Yee et al. (2021)
	Ulcerous gastritis	*Sulcascaris sulcata*	Marcer et al. (2020)

Therefore, prioritizing vaccination as a preventive measure aligns with the goals of promoting animal welfare, enhancing productivity, and safeguarding public health within the animal farming industry.

Vaccines serve as invaluable tools in enhancing and strengthening immune responses within organisms. They work by stimulating the immune system to produce a targeted response, specifically through the generation of antibodies that provide immunity against particular pathogens (Tammas et al. 2024). By introducing a harmless component or a weakened form of the pathogen, vaccines effectively train the immune system to recognize and combat the actual disease-causing agents should they encounter them in the future (Tammas et al. 2024). These biological products can take various forms, including live-attenuated vaccines containing weakened pathogens, subunit vaccines comprising antigenic components of pathogens, or innovative approaches like mRNA vaccines and DNA vaccines, which encode specific antigens (Ghattas et al. 2021; Cid and Bolívar 2021). The administration of vaccines to animals aims to fortify their resilience and resistance against targeted diseases, ultimately reducing mortality rates and enhancing overall productivity within the animal farming industry (Hu et al. 2020). Despite the significant benefits of vaccination, conventional methods, such as administering individual injections, present a range of challenges. These traditional approaches are often labor-intensive and time-consuming, requiring considerable effort and resources to deliver each vaccine dose to individual animals or individuals (Ghattas et al. 2021). The process involves precise execution, and a substantial amount of time dedicated to ensuring that each recipient receives the correct dosage. This can be particularly demanding in large-scale settings or for populations with a high vaccination coverage requirement. Additionally, the need for trained personnel and the logistical complexities associated with maintaining proper vaccine storage and handling further contribute to the overall burden of these conventional vaccination methods (Ghattas et al. 2021). As a result, there is an ongoing need for more efficient and scalable vaccination strategies to streamline the process and improve overall effectiveness in disease prevention. Among these alternatives, edible vaccines emerge as a promising solution. By incorporating vaccine components into edible materials, such as feed, the process becomes more streamlined and accessible (Tammas et al. 2024). Edible vaccines offer the potential to revolutionize mass vaccination efforts in animal farming, offering a practical and efficient means of disease prevention while minimizing logistical hurdles (Mičúchová et al. 2022). As research in this area advances, the integration of edible vaccines into animal husbandry practices holds promise for enhancing disease management strategies and promoting sustainable agricultural practices.

Edible vaccines

Edible vaccines represent a promising innovation in the farming industry, offering significant advantages in efficiency and convenience. By integrating vaccination and feeding processes, farmers can save considerable time and effort. Many edible vaccines currently under development utilize transgenic plants that have been genetically engineered to express specific antigenic components of pathogens. These specially modified plants are designed to produce proteins or other molecules that mimic the structures of disease-causing agents. When consumed, these plants can stimulate the immune system to recognize and respond to these antigens, thereby inducing the production of antibodies (Sahoo et al. 2020). In addition to the ease of administration, plant-based edible vaccines offer several notable advantages over conventional vaccines. One significant benefit is their improved long-term storage capabilities (Burnett and Burnett 2020). Unlike traditional vaccines that often require stringent refrigeration and handling to maintain their efficacy, plant-based vaccines can be stored at ambient temperatures, which reduces the risk of spoilage and contamination (Burnett and Burnett 2020). This attribute not only enhances the stability of the vaccines but also simplifies the logistics and distribution, particularly in resource-limited settings. Plants have natural mechanisms for preserving their proteins and other compounds. Horn et al. (2004) showed that transformed plants producing heterologous proteins could still accumulate the protein even after the plant biomass had dried. By expressing antigens in plants, these proteins can be stored within the plant tissues, which can help maintain their stability and activity over time. Plants can also be processed into dried forms, which further extends the shelf life. Moreover, the plants offer excellent scalability because they can be cultivated practically anywhere (Obembe et al. 2011). The production costs for plant-based vaccines are generally lower compared to those for conventional vaccines (Rosales-Mendoza et al. 2020). This cost advantage can make vaccines more accessible and affordable, especially in underserved regions. Additionally, plant-based systems have the potential for more complex protein expressions (Burnett and Burnett 2020). Plants can be engineered to produce a variety of proteins with intricate structures that may be difficult to achieve using other production methods. Plant cells have been shown to express fully functional antibodies, including the necessary post-translational modifications (PTMs) (Diamos et al. 2020; Nessa et al. 2020). Diamos et al. (2020) reported that the efficiency of antibody production was 1.5 g/kg of leaf tissue. Although PTMs are currently given low priority in conventional vaccine manufacturing, they are believed to enhance a vaccine's potential and immunogenicity, making it more effective and efficient (Ojha and Prajapati 2021). This capability allows for the development of vaccines with enhanced immunogenic properties, potentially leading to more effective and diverse vaccine formulations.

Along with their ability to produce more complex and larger proteins, plants can also be utilized in vaccine development and manufacturing due to their capacity for PTMs. This is advantageous in vaccine development because certain moieties integrated by pathogenic organisms are part of their pathogenic mechanisms and contribute to immunogenicity (Watanabe et al. 2019). For example, O-glycosylated proteins play a significant role in antigen display in antigen-presenting cells and the general major histocompatibility complex (MHC)-based immune response (Ojha and Prajapati 2021). Previous discussions have shown that a glycosylated protein produced in a higher-level expression system can induce greater immunogenicity compared to a non-glycosylated protein produced in a bacterial expression system (Feng et al. 2022; Nascimento and Leite 2012; Ojha and Prajapati 2021). Due to the high complexity and diversity of PTMs (Friso and van Wijk 2015), genetically engineered plants, including duckweeds, are capable of producing proteins that mimic the PTMs found in pathogens, potentially leading to more effective vaccines. Additionally, plant-based glycans, such as those resulting from N-glycosylation, may generally enhance vaccine development due to their increased immunogenicity. Specifically, plant-based glycans could improve the detectability of pathogenic antigens, particularly through lectins or mannose/fucose receptors on the surface of dendritic cells (Franconi et al. 2010). This capability allows for the development of vaccines with enhanced immunogenic properties, potentially leading to more effective and diverse vaccine formulations. Overall, these benefits contribute to making plant-based edible vaccines a promising and innovative approach in the field of immunization.

Notably, edible vaccines primarily activate the mucosal immune system of vaccinated animals, which encompasses both innate and adaptive immunity mechanisms (Debnath et al. 2022). Within the gastrointestinal tract, antigenic proteins delivered by edible vaccines are primarily captured by microfold (M) cells, specialized epithelial cells found in the follicle-associated epithelium of Peyer's patches (Kurup and Thomas 2020). M cells possess the unique ability to transport macromolecules from the intestinal lumen to antigen-presenting cells within Peyer's patches, facilitating the initiation of immune responses (Clark et al. 2001). This targeted delivery mechanism enhances the efficacy of edible vaccines, contributing to their potential as a practical and effective tool for disease prevention in animal farming. Tobacco has emerged as a prominent candidate for edible vaccine platforms, with transgenic tobacco varieties being developed to express vaccines against various diseases (Kurup and Thomas 2020). Notably, Dow AgroScience LLC has produced transgenic tobacco expressing a vaccine against Newcastle disease, which has received approval from the United States Department of Agriculture (USDA) (Takeyama et al. 2015). Similarly, Planet Biotechnology Inc. has developed transgenic tobacco expressing a secretory antibody vaccine targeting tooth decay, approved by the European Union (EU) (Kim and Yang 2010). However, despite its utility in vaccine production, tobacco is not suitable for animal feed, prompting the exploration of alternative platforms. Duckweeds, members of the *Lemnoideae* family, have garnered attention as promising alternatives. These small monocots, typically floating on water surfaces, offer unique advantages. With frond sizes ranging from 0.5 to 4 mm, duckweeds are highly adaptable and possess rapid growth rates (Yang et al. 2021; Yoshida et al. 2021). The *Lemnoideae* family comprises five identified genera: *Landoltia*, *Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella* (Sembada and Faizal 2019). This diversity underscores the potential of duckweeds as versatile platforms for both animal feed and the production of value-added bioproducts (Fig. 1). Exploring the dual-functionality of duckweeds could unlock novel opportunities for sustainable agriculture and biotechnology, offering solutions that integrate nutrition, disease prevention, and production efficiency in animal farming practices.

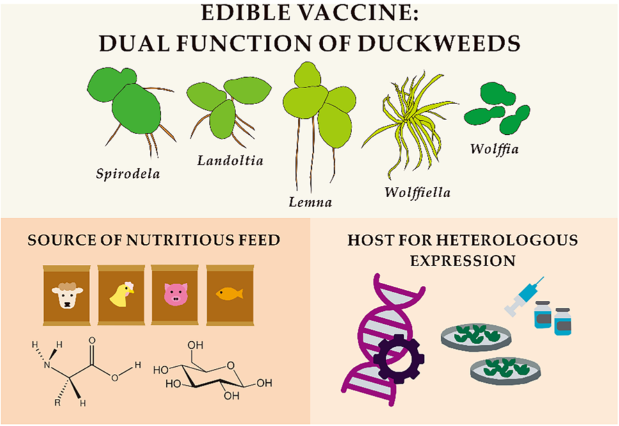

Fig. 1 The dual function nature of duckweeds as feed sources and producers of value added bioproducts. Duckweed species commonly used as feed include those from the genera *Spirodela*, *Landoltia*, *Lemna*, *Wolffia*, and *Wolffia*. Each of these has been shown to possess high levels of essential nutrients, including but not limited to essential amino acids, starch, fatty acids, minerals, carotenoids, tocopherols, and sterols. In addition to being excellent feed sources, duckweeds are also an outstanding platform for heterologous protein production due to their ease of manipulation, rapid growth, and ability to accumulate proteins

Duckweeds as nutritious feed sources

Duckweeds, characterized by their small size and aquatic habitat, offer a plethora of advantages that make them highly favorable for various applications, including animal feed production. With frond sizes ranging from 0.5 to 4 mm, depending on the genus, duckweeds exhibit remarkable growth rates, with *Wolffia* doubling its biomass every 3.28 days at a specific growth rate of 0.21/day (Faizal et al. 2021; Sembada and Faizal 2023). This rapid growth, coupled with their ability to accumulate proteins and starch in substantial amounts—up to 36.2% and 17.2% dry weight, respectively—renders duckweeds an excellent source of nutrition (Li et al. 2016). Moreover, research indicates that duckweeds offer additional health benefits for farm animals. For instance, studies have shown that incorporating duckweeds into the diets of laying hens results in eggs with higher Omega-3 levels, enhancing their nutritional value (Anderson et al. 2011). Similarly, feeding duckweeds to dairy cows has been demonstrated to improve the hematological and antioxidant profiles of their blood plasma, indicating potential health benefits (Tanuwiria and Mushawwir 2020). Notably, duckweeds boast an impressive nutritional profile, containing all essential amino acids at higher levels than several traditional feed sources such as chickpea, corn, lentil, rice, soybean, and wheat (Appenroth et al. 2018). Additionally, duckweeds are rich in sugars in the form of starch, comprising 18–53% of their dry weight, making them an essential component of animal feed formulations (Xu et al. 2023). Furthermore, duckweeds contain a variety of other compounds beneficial for animal health, including fatty acids, minerals, carotenoids, tocopherols, and sterols (Appenroth et al. 2018; Sembada and Faizal 2022). This comprehensive nutritional profile underscores the potential of duckweeds as a sustainable and versatile feed source for enhancing animal health and productivity in the agricultural sector.

Duckweeds boast a diverse array of fatty acids, with polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), and monounsaturated fatty acids (MUFA) being the most prevalent (Appenroth et al. 2018). Within these groups, various individual fatty acids are found in duckweeds, ranging from capric acid (C10:0) to montanic acid (C28:0), with each contributing to the nutritional profile of these plants. In addition to fatty acids, duckweeds contain essential minerals such as calcium, potassium, magnesium, sodium, phosphorus, as well as microelements like iron, manganese, copper, zinc, and iodine. They also provide carotenoids such as (all-E)-β-carotene, (9Z)-β-carotene, and tocopherols like α-tocopherol, which contribute to their nutritional value. Furthermore, sterols found in duckweeds include phytol, campesterol, and stigmasterol, among others (Appenroth et al. 2018). These compounds, coupled with the ease of cultivation and low cost of production, make duckweeds an exceptional source of nutrition for animals. Several studies have demonstrated the added benefits of incorporating duckweeds into animal feed formulations, highlighting their potential to enhance animal health and productivity (see Table 2 for details). Overall, the comprehensive nutritional profile and versatility of duckweeds make them a promising and sustainable feed option for the agricultural industry.

Duckweeds as edible vaccine platforms

The expression of heterologous proteins via genetic engineering of plant genomes offers numerous advantages over other expression systems (Kulshreshtha et al. 2022). Notably, plant-based production boasts low production costs, scalability, the capacity to synthesize complex proteins, and a reduced risk of contamination by human or animal pathogens (Gerszberg and Hnatuszko-Konka 2022). As genetic engineering continues to advance, an increasing number of plant species are being utilized as platforms for heterologous protein production (Sembada et al. 2024). Among these platforms, duckweeds have emerged as a promising option. Duckweeds offer several characteristics that make them highly suitable for heterologous protein production. They can be easily transformed using *Agrobacterium*, and they can be induced into callus, which can then be regenerated back into fronds (Li et al. 2004; Yamamoto et al. 2001). This versatility facilitates efficient genetic modification and protein expression in duckweeds, as previously reported in several studies (Fig. 2). Indeed, transgenic duckweeds have demonstrated the ability to produce various valuable bioproducts, including hirudin (Kozlov et al. 2019), highlighting their potential as a platform for heterologous protein expression. As research in this field progresses, duckweeds are likely to become increasingly important for the production of heterologous proteins. Their ease of manipulation, rapid growth, and ability to accumulate high levels of proteins make them attractive candidates for industrial-scale protein production. By harnessing the capabilities of duckweeds, researchers can explore new avenues for the production of valuable bioproducts with diverse applications.

The genomes of duckweed species have been extensively investigated and sequenced (An et al. 2018), with the National Centre for Biotechnology Information (NCBI) hosting a growing database of duckweed sequences. This resource not only aids genetic engineering endeavors but also enhances evolutionary and phylogenetic analyses. Duckweeds' versatility as heterologous protein production platforms underscore their potential in the animal farming industry, particularly in the production of edible vaccines.

Table 2 Added benefits of duckweeds as feed include enhanced nutrition profiles

Animals	Feed types	Effects	Refs
Birds: Japanese quail (*Coturnix japonica*)	Supplementation of *W. globosa* (25–75%) and *W. arrhiza* (0–20%) in the dietary meals	Increased skin pigmentation and color intensity of the yolk	Chantiratikul et al. (2010; Suppadit et al. 2012)
Chickens: *Gallus domesticus* Linn	Supplementation of *L. minor* in chicken fed normal diet	Increased body weight and lowering the cost of meat production	Paguia et al. (2022)
Chickens: Star Cross Brown laying hen	Sun dried of *L. minor* in poultry diet (0 – 150 g/kg dietary level)	Increased the pigment of egg yolk produced	Akter et al. (2011)
Chickens: Vencobb broiler	Replacing dietary sesame oil cake (SOC) by *L. minor* in the diets	Partial replacement of SOC by duckweed increased profitability	Ahammad et al. (2003)
Dogs	Incorporating *L. punctata* into dog diets at 10–30%	Dogs produced firmer stools but no significant effect on palatability	Brown et al. (2013)
Ducks	Giving duckweed in dry-mash, wet form (slurry) or fresh biomass	Feeding with fresh biomass gave the best egg yolk pigmentation	Indarsih and Tamsil (2012)
Ducks: Jinding	Partial replacement of mustard oil cake with *L. perpusilla* as protein supplement in diets	Significantly increased the egg production and profitability	Khandaker et al. (2007)
Fishes: African Mud Catfish (*Clarias gariepinus*)	Supplementation of *L. pauciscostata* in the juvenile diets	Gave the best benefit cost ratio (BCF)	Sogbesan et al. (2015)
Fishes: Grass carp (*Ctenopharyngodon idella*) and Silver carp (*Hypophthalmichthys molitrix*)	Supplementation of *L. minor* as major ingredients in plant based diets	Comparable to the soybean meal in the term of growth rate and feed conversion efficiency	Aslam et al. (2017)
Fishes: Indian major carp (*Labeo rohita*)	Supplementation of sundried *L. minor* powder in the meal diets	Gave significant higher weight gain and fish growth rate	Kaur et al. (2012)
Goats: West African dwarf	Fresh *S. polyrrhiza*	Highest N-retention in the goats compared to grass fed	Babayemi et al. (2006)
Swine	Lemna protein concentrate (68%) extracted from de-oiled and dehydrated biomass	Greater standardized total tract digestibility compared with fish meal and soybean meal	Rojas et al. (2014)
Swine: Large White x Mong Cai	Incorporating of 5% of *L. minor* in the diet dry matter	Gave the stimulating effect on live-weight gain	Hang (1998)
Sheeps: Fine-Wool Merino (*Ovis aries*)	Supplementation of sun-dried duckweed (50–100 g/d) in the diets	Increased wool growth rate	Nolan et al. (2001)
Shrimps: Pacific white (*Litopenaeus vannamei*)	Supplementation of *Lemna* sp. flour (0–35%) on the fishmeal	Growth performance of shrimps was significantly better	Flores-Miranda et al. (2015)
Turtles: *Trachemys scripta*	Fresh *L. valdiviana* and *L. minor* biomass	Served as plant diet as complement to animal diet due to the omnivorous nature of turtles	Bouchard et al. (2010)

Recent studies have confirmed duckweeds' ability to express antigenic proteins, eliciting immune responses in animals and conferring immunity against pathogens (Bertran et al. 2015). Notably, research has targeted several significant pathogens and diseases (Fig. 3), including avian influenza, tuberculosis, porcine epidemic diarrhea, mastitis, Newcastle disease, hemorrhagic disease, and vibriosis. These findings highlight the promising role of duckweeds in disease prevention within animal populations and underscore their potential as a sustainable solution for enhancing animal health and productivity in agricultural settings.

It is well established that duckweeds are among the fastest-growing plant species, capable of producing a higher yield per area compared to average crop plants. Depending on the species, duckweeds have been shown to achieve a doubling time of 1.34 to 4.54 days ($\mu = 0.153–0.519$/day) (Faizal et al. 2021; Sembada et al. 2019). These doubling times are comparable to those of Human Embryonic Kidney (HEK) 293 cells (1.38 days) and Chinese Hamster Ovary (CHO) cells (0.6–1 day) (Ritacco et al. 2018; Abaandou et al. 2021) and exceed those of important crop plants considered by the Food and Agriculture Organization of the United Nations (FAO) and USDA (Ziegler et al. 2015). Consequently, duckweeds are also known for their higher protein yields per area. These higher protein yields have been observed in comparison to several other crops, including soybean, rice, and corn (Baek et al. 2021). Nevertheless, efforts are ongoing to further increase both biomass and

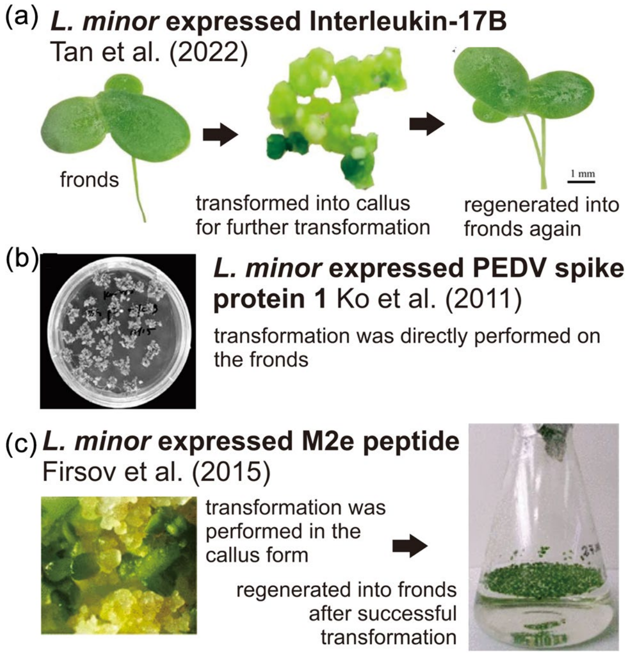

Fig. 2 Genetic modification and protein expression in duckweeds reported in several studies. A significant number of previous studies have investigated the potential and capabilities of duckweeds in the manufacturing of heterologous proteins. Three notable examples are **A** Tan et al. (2022), **B** Ko et al. (2011), and **C** Firsov et al. (2015). **A** Tan et al. successfully engineered *L. minor* to express chicken interleukin-17B by inducing the fronds to form callus, which was then incubated with *A. tumefaciens* strain GV3101 containing pCAMBIA2301. The callus was subsequently regenerated into fronds through the regulation of growth factors. **B** Ko et al. managed to transform *L. minor* to express the spike protein of porcine epidemic diarrhea virus (PEDV) tagged with c-myc to facilitate the purification process. The transformation was performed without first inducing the fronds to form callus. **C** Firsov et al. transformed *L. minor* to produce the M2e protein of avian influenza virus H5N1 by introducing a cassette containing the M130 gene sequence using *A. tumefaciens*

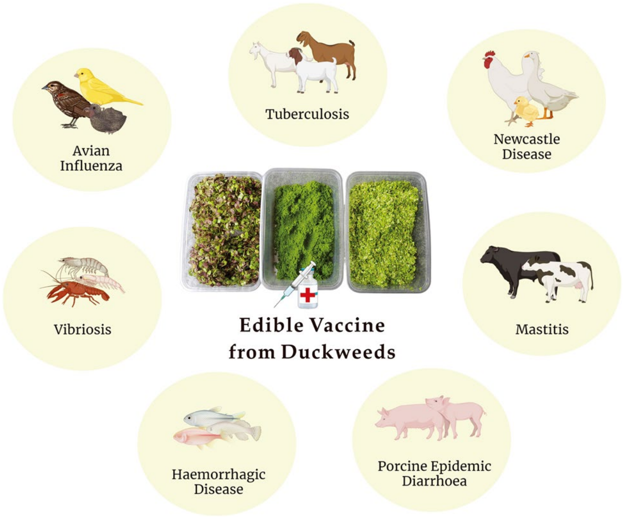

Fig. 3 Application of duckweeds-based edible vaccines against several diseases in the animal farming industry. Research efforts on duckweed-based edible vaccines have been concentrated on preventing diseases affecting various animals. Examples include avian influenza, tuberculosis, porcine epidemic diarrhea, mastitis in dairy animals, Newcastle disease in birds, hemorrhagic diseases in fish, and vibriosis in aquatic farm animals

protein yields, involving genetic engineering, environmental manipulations, and their combinations. Environmental manipulations aim to introduce stresses or, conversely, optimize growth conditions to induce specific metabolic processes. For example, growing duckweeds under different light intensity conditions has been shown to affect biomass yield (Femeena et al. 2023). Other environmental parameters include nutrient availability, temperature, air circulation, photoperiod, humidity, and heavy metal concentrations, among others (Coughlan et al. 2022; Femeena et al. 2023).

As discussed above, duckweeds offer exceptional ease of transformation, which benefits both heterologous protein production and other applications. Common genetic engineering approaches include enhancing transcription and translation and downregulating transcriptional and post-transcriptional silencing genes (Feng et al. 2022). For example, one method of regulating extraneous genes is the use of specific promoters. In duckweeds, as in other plant species, the 35S promoter derived from *Cauliflower mosaic virus* (CaMV) is one of the most commonly used promoters for constitutive expression of transgenes (Amack and Antunes 2020; Feng et al. 2022). However, recent advances in duckweed biotechnology have identified a constitutive endogenous promoter, *LpSUT2*, which maintains activity better than *CaMV35S* in the presence of antibiotics (Wei et al. 2024). In addition to constitutive traits, genetic engineering can introduce inducible traits through the use of inducible promoters, combining genetic engineering and environmental manipulation to elicit specific traits. An example involving duckweeds is the use of *MmDGAT2*, an estradiol-inducible promoter, for the production of triacylglycerol (Liang et al. 2023).

The introduction of a foreign protein may lead to changes in the balance of endogenous proteins, potentially reducing the concentration of some essential proteins while increasing the overall protein content due to the presence of the recombinant protein (Burnett and Burnett 2020). Recombinant protein expression could also alter the overall protein content of duckweeds. Tan et al. (2022) expressed interleukin-17B in *L. minor*. This protein reached a concentration of up to 1.89 µg/g, which corresponds to 0.036% of the total soluble protein. In another study, the heterologous expression level reached up to 0.24% of the total soluble protein (Sun et al. 2007). If recombinant proteins represent a small portion of the total soluble proteins, the overall balance of essential nutrients in duckweeds, such as amino acids, vitamins, and minerals, is likely preserved. This means that the nutritional quality of duckweeds as a feed source remains largely intact. Recombinant protein expression could also impact the levels of secondary metabolites in plants. Secondary metabolites, which include vitamins, flavonoids, and phenolics, might be reduced or altered due to the metabolic demands of recombinant protein production. Yao et al. (2022) reported the accumulation of saponins following the heterologous expression of the phenylalanine ammonia lyase gene. In another study, the expression of the transcription factor, *LtP1L*, in *L. turionifera* could alter the flavonoid content (Wang et al. 2024).

When comparing antigen production productivity across different platforms, duckweeds generally offer a lower antigen yield per production time compared to conventional systems. Duckweeds, due to their rapid growth and high biomass yield (Faizal et al. 2021), provide a cost-effective and scalable platform, but their overall antigen productivity might not match that of bacteria or mammalian cells. Firsov et al. (2015) reported that the expression of the avian influenza virus antigen in *L. gibba* yielded up to 40 µg/g of plant fresh weight. Bacteria, particularly *E. coli*, can produce recombinant proteins in large quantities relatively quickly, often achieving milligrams to grams per liter of culture within days (Aguilar-Yáñez et al. 2010), though they may struggle with complex proteins requiring PTMs. The expression of the avian influenza virus antigen in *E. coli* could achieve average overall yields of 0.5–1.0 g/L (Aguilar-Yáñez et al. 2010). In contrast, mammalian cell systems, such as CHO cells, can produce complex, properly folded proteins with PTMs at high yields, typically in the range of grams per liter of culture (Chen et al. 2019), but this process is more costly and time-consuming, often taking several weeks. Chen et al. (2019) reported that the expression of the avian influenza virus antigen in CHO cells yielded 18–20 mg/L. Therefore, duckweeds still have the potential to be developed as a platform for antigen expression and could serve as a promising dual-function platform for both feed and vaccine production as also presented in Table 3. Table 3 is a subset of Table 4 and shows the instances in which duckweeds have been used as hosts for the production of antigenic proteins. The studies clearly demonstrate the success of duckweed-based edible vaccines in eliciting proper immune responses in test subjects. They therefore serve as exemplars of duckweeds' potential in the production of edible vaccines.

At the time of writing, only a few plant-produced vaccines have been approved. Notable examples include the vaccine against Newcastle disease, which was the first plant-made vaccine approved in 2006 (Takeyama et al. 2015), and more recently, the Covifenz vaccine against COVID-19 registered in Canada (Su et al. 2023). This limited number of approved plant-produced vaccines reflects the current state of the art in plant expression systems as platforms for heterologous

Table 3 Previous studies showcasing the capability of duckweeds as a promising dual-function platform for both feed and vaccine production

Species	Target pathogen	Expressed protein	Test subject	General results	Refs
L. minor	H5N1	H5	Chickens	Up to 100% survival rate against H5N1	Bertran et al. (2015)
W. globosa	*V. alginolyticus*	LamB	Zebrafish	63.3% relative percent survival against *V. alginolyticus*	Heenatigala et al. (2020)
L. minor	H5N1	M2e fused to Ricin Toxin B	Mice	IgG against M2e detected	Firsov et al. (2018)

Table 4 Heterologous proteins expressed in duckweeds

Proteins	Hosts	Transformation results	Refs
Anti-tumour necrosis factor alpha (TNFα)	*A. tumefaciens* transformation in *S. punctata* with pCAMBIA1304 plasmid	Protein expression levels of up to 6.3% of total soluble protein	Balaji et al. (2016)
Aprotinin	*A. tumefaciens* transformation in *S. oligorrhiza* with pBIN + plasmid	Expression levels up to 3.7% of water soluble proteins were detected in the plant	Rival et al. (2008)
Endoglucanase E1	*A. tumefaciens* transformation in *L. minor* with pCel25IX plasmid	Expression level of the enzyme was up to 0.24% of total soluble protein	Sun et al. (2007)
Enhanced yellow fluorescent protein (eYFP)	*A. tumefaciens* transformation in *S. polyrhiza* turions with pB7YWG2 plasmid	Turions ability to withstand harsh conditions enhances the versatility of the transformant	Chanroj et al. (2021)
Hirudin	*A. tumefaciens* transformation in *L. minor* with pBI121-hir plasmid	The maximum hirudin accumulation was equal to 0.02% of the total soluble protein	Kozlov et al. (2019)
Hirudin	*A. tumefaciens* transformation in *W. arrhiza* with pCamHIR plasmid	The maximum accumulation of recombinant hirudin was 775.5 ± 111.9 ng/g of fresh weight of the plant	Khvatkov et al. (2021)

protein expression on a commercial scale. In comparison to bacterial cells, plant cells generally have a longer doubling time, which leads to lower overall product yields. Historically, most therapeutic proteins were developed with the need for humanized PTMs in mind, making mammalian cells the logical choice for production. Consequently, this focus has driven extensive advancements in the utilization and engineering of bacterial and mammalian expression systems, particularly for the manufacturing of heterologous proteins. Supporting systems for these platforms have also advanced significantly in recent decades, resulting in increased robustness, cost-effectiveness, and compliance with regulatory standards (Schillberg et al. 2019).

Inevitably, the same level of effort has not been applied to the development of plant expression systems, resulting in their underdevelopment and limited utilization compared to conventional platforms. This underdevelopment is evident not only in the biotechnological aspects of protein manufacturing but also in engineering aspects such as downstream processing (Schillberg et al. 2019). This has, in turn, discouraged further development in this area, perpetuating a cycle of underdevelopment and stagnation. The currently established technologies in plant-based protein production still have significant drawbacks compared to more conventional platforms, such as lower yields and less humanized PTMs. However, the use of duckweeds as an expression platform could address the concern of low growth rates, as duckweeds are among the fastest-growing plants, even comparable to HEK293 cells. Additionally, while humanized PTMs are challenging to achieve, their necessity is less critical in vaccine manufacturing compared to the PTMs of pathogens, which are simpler and more feasible to produce. Despite these challenges, extensive research is ongoing in the production of therapeutic proteins for both human applications and other uses.

Duckweeds as edible vaccine platform for avian influenza

Avian influenza, commonly known as bird flu, is a contagious disease that primarily affects birds raised on farms, including chickens, turkeys, ducks, and geese (Palmore 2006). It is caused by viruses belonging to the *Orthomyxoviridae* family, classified into influenza A, B, and C viruses based on their hemagglutinin (HA) and neuraminidase (NA) proteins (Nuwarda et al. 2021). While most avian influenza viruses do not infect humans, certain strains, such as H5N1, H7N7, and H9N2, have demonstrated zoonotic potential (Kessler et al. 2021). As such, avian influenza viruses pose a significant threat to both animal and human health, underscoring the urgent need for effective vaccines. Vaccination is crucial for mitigating the spread of the disease and preventing potential outbreaks in both poultry and humans. Developing effective avian influenza vaccines is essential for safeguarding public health and ensuring the sustainability of poultry farming practices.

In recent studies, researchers have explored the potential of duckweeds as a platform for expressing candidate proteins, with a particular focus on the hemagglutinin (HA) protein. Bertran et al. (2015) conducted experiments wherein they expressed the haemagglutinin H5 protein from clade 2.1.1 A/chicken/Indonesia/7/2003 (Indo/03) in *L. minor*, utilizing the Lemna Expression System (LEX System™, Biolex Therapeutics, Pittsboro, NC). Through Western blot analysis, they confirmed successful expression of the H5 protein, with a size of 77 kDa, and estimated its expression to reach 12% of the total dissolved protein, approximately 280 mg/kg of frozen biomass. To evaluate the efficacy of the edible vaccine produced, white leghorn chickens infected with three different types of viruses were tested: clade Indo/03 virus (the same seed virus with antigen originating from), clade 2.3.2.1 A/chicken/Vietnam/NCVD-421/2010 (VN/10) virus, and clade 2.1.3.2 A/chicken/West Java/PWT-WIJ/2006 (PWT/06) virus. Results indicated varying survival rates among the chickens following virus infections (Bertran et al. 2015). Chickens infected with Indo/03 showed full protection, primarily attributed to the virus used in the edible vaccine's antigen source. These findings highlight the potential of duckweed-based edible vaccines in providing protection against avian influenza viruses and warrant further investigation into their effectiveness and applicability.

In a study by Nguyen et al. (2012), the expression of the avian influenza hemagglutinin (AIV HA) gene was successfully achieved in isoleucine-auxotrophic *L. minor* 8627. This isoleucine auxotrophy was induced through RNA interference (RNAi) targeting the threonine deaminase (TD) gene. Two vectors, AUXC01 and AUXC02, both containing *L. minor* TD cDNA (LmTD), were utilized to produce auxotrophic isoleucine plants. These vectors were driven by constitutive promoters, namely the Superpromoter and *S. polyrhiza* polyubiquitin (SpUBQ), respectively. The AIV HA gene sequence, sourced from the A/chicken/Indonesia/7/2003 H5N1 virus isolate, was inserted into three different vectors: MERB05, MERB06, and MERB07. The results demonstrated successful gene silencing of the TD gene and simultaneous expression of the AIV HA gene in *L. minor*. Moreover, there was a notable increase in AIV HA expression, robust auxotrophic phenotype, and the plants showed full recovery post-isoleucine supplementation. These findings underscore the feasibility of utilizing genetic engineering techniques to express foreign genes in duckweeds, paving the way for potential applications in vaccine production and biotechnology. Further research is warranted to explore the full potential of this approach in agricultural and medical contexts.

In another study by Thu et al. (2015), the expression of H5N1 HA1 protein was demonstrated in *S. polyrhiza*. The transformation process involved the use of *A. tumefaciens* AGL-1 carrying the p6D35S plasmid containing the HA1 gene. PCR analysis confirmed successful transgene insertion, with the expected band position observed at approximately 600 bp for the HA1 gene. Given the potential for antigenic shifts and drifts in influenza viruses, efforts have been made to develop a more universal avian influenza vaccine. One such candidate is the M2e (extracellular domain of the matrix protein 2) peptide-based vaccine, which exhibits greater conservation across avian influenza virus strains (Zhao et al. 2010). By targeting more conserved antigens, researchers aim to create vaccines capable of providing broader protection against diverse strains of avian influenza viruses, thus addressing challenges posed by viral evolution and variability.

In a study by Firsov et al. (2015), M2e protein expression was achieved in *L. minor* through *Agrobacterium*-mediated gene transfer. A transformation cassette containing the M130 gene sequence for M2e peptide expression was developed and introduced into *L. minor* callus via *A. tumefaciens* mediation, followed by regeneration into fronds. Various analyses, including GUS assays, PCR, Southern blot, Western blot, and ELISA, confirmed successful production of the M2e protein by transgenic *L. minor*, reaching a titre of 0.97 mg/g frozen weight. Despite successful protein expression, initial trials on mice failed as they did not consume the transgenic duckweed. Consequently, protein extracted from the biomass was administered to the mice, revealing a specific immune response against M2e elicited by the transformed duckweed protein. This suggests the potential of duckweeds as heterologous protein expression systems, particularly for avian influenza virus antigens. These findings underscore the promise of duckweeds in biotechnological applications, offering a sustainable and cost-effective approach to vaccine production and disease prevention in agricultural and medical fields.

Duckweeds as edible vaccine platform for tuberculosis

Tuberculosis (TB) is an infectious disease affecting both humans and animals, caused by bacteria of the genus *Mycobacterium*. Swine, rabbits, and cattle are particularly susceptible to TB infections among farm animals (Sevilla et al. 2020). Certain mycobacterial species, including *Mycobacterium bovis*, are zoonotic, posing a threat to both animal and human health. *M. bovis* primarily infects cattle and can induce TB-like symptoms in humans and other mammals (Damene et al. 2020). Due to its ability to cross species barriers, *M. bovis* infections in cattle can have significant implications for public health, particularly in regions where consumption of unpasteurized dairy products is common. Therefore, effective surveillance and control measures are essential to prevent the transmission of TB from animals to humans and to mitigate the spread of this infectious disease in both animal and human populations.

In the quest for effective vaccines against mycobacterial infections, numerous mycobacterial antigens have been investigated as potential candidates. Among these, the Early Secreted Antigenic Target 6 (ESAT6), encoded by the esxA gene, and Antigen 85B (Ag85B), encoded by the fpbBTMD gene, have garnered considerable attention. In a study by Peterson et al. (2015), *L. minor* was genetically modified to express a fusion protein ESAT6-Ag85B(ΔTMD)-6His. The genetic engineering process involved the use of *A. rhizogenes*, a commonly used *Agrobacterium* strain known for inducing hairy root cultures. The transformation cassette, constructed on the pCB064 vector, contained the 35S promoter and genes necessary for recombinant fusion protein production. The infection process between plant tissue and *A. rhizogenes* suspension lasted for 30 min. Confirmation of transgene integration through PCR analysis targeting rolB, nptII, and fbpBTMD genes indicated successful transformation (Matvieieva et al. 2011). These findings underscore the potential of duckweeds as versatile platforms for heterologous protein expression, paving the way for the development of novel vaccines against mycobacterial infections.

Duckweeds as edible vaccine platform for Newcastle disease

Newcastle disease (ND) is a highly contagious viral infection that primarily afflicts birds, particularly domestic poultry and wildfowl. The causative agent, the Newcastle disease virus (NDV), belongs to the *Paramyxoviridae* family (Rtishchev et al. 2023). ND poses a significant threat to global poultry populations, leading to substantial economic losses and concerns for food security. Transmission primarily occurs through direct contact with infected birds' bodily fluids, including respiratory secretions, feces, and contaminated feed and water sources (Dzogbema et al. 2021). In poultry, NDV infection can result in severe inflammation, particularly with virulent strains such as genotype VII or "GM" strains (Gao et al. 2022). These strains elicit robust immune responses characterized by the production of proinflammatory cytokines, including interleukin-1β (IL-1β). IL-1β serves as a pivotal proinflammatory cytokine in the innate immune response against NDV (Cai et al. 2023). Immune cells detect the presence of the virus and release IL-1β, orchestrating the inflammatory cascade. This cytokine plays a crucial role in coordinating the immune response by recruiting and activating immune cells, notably macrophages and neutrophils, to the site of infection (Duque and Descoteaux 2014). Through its actions, IL-1β helps to contain viral spread and initiate the adaptive immune response, ultimately contributing to the resolution of NDV infection in affected birds. Understanding the mechanisms underlying the immune response to NDV is essential for developing effective control strategies and vaccines to combat this devastating poultry disease.

Tan et al. (2022) successfully expressed chicken interleukin-17B (chIL-17B) in *L. minor*. Initially, plant explants were induced to form callus using growth regulators on MS media. Subsequently, the calluses were incubated with *A. tumefaciens* strain GV3101 containing pCAMBIA2301(p2301). Transformants were selected based on GUS staining and PCR analysis using specific primers. Protein expression was assessed via Western blot analysis with anti-His-Tag monoclonal antibody, and the recombinant IL-17B was purified using Ni–NTA agarose (Tan et al. 2022). Furthermore, they demonstrated that duckweed-based chIL-17B, when used as an adjuvant, effectively enhanced systemic and mucosal immune responses, particularly elevating mucosal sIgA levels at effector sites, and reducing virus load (Tan et al. 2022). These findings underscore the potential of duckweed-based platforms for the production of immunomodulatory proteins and their application as adjuvants in vaccine development, highlighting the importance of such strategies in enhancing immune responses against infectious diseases.

Duckweeds as edible vaccine platform for mastitis

Mastitis, a prevalent and economically consequential ailment in dairy animals, predominantly afflicts cattle but can also impact goats and sheep. This inflammatory malady of the mammary gland is typified by infection and swelling, often induced by various microorganisms, primarily bacteria (Cobirka et al. 2020). The disease manifests in both clinical and subclinical forms, with bacteria such as *Staphylococcus aureus*, *Streptococcus* spp., and *E. coli* among the culprits (Ashraf and Imran 2020). Clinical mastitis presents observable indications like udder swelling, heat, redness, and tenderness, with milk from affected quarters exhibiting abnormalities such as clots, pus, or blood (Samad 2022). Conversely, subclinical mastitis may lack overt external signs but is discernible through elevated somatic cell counts in milk, indicating an ongoing infection. Effective management and timely intervention are crucial in curbing mastitis's detrimental effects on animal health and productivity. Implementing rigorous hygiene protocols, employing mastitis control strategies, and promptly treating infections are essential steps in mitigating the impact of this pervasive disease on dairy operations.

Penaeidins 3a, vital antimicrobial peptides present in the hemolymph of shrimp and other crustaceans like penaeid shrimp, constitute an integral component of the shrimp's innate immune system, serving as a defense mechanism against bacterial and fungal infections (Aweya et al. 2021). These peptides have garnered attention for their potent antibacterial activity against notorious pathogens such as *S. aureus* and *E. coli* (Xiao et al. 2021; Wu et al. 2019). In a recent study by Yang et al. (2023), Penaeidins 3a (Pen3a) expressed in *L. turionifera* 5511 exhibited notable antibacterial efficacies against both *E. coli* and *S. aureus*. The transformation process involved the introduction of the Pen3a expression vector pCAMBIA-1301-Pen3a into *L. turionifera* 5511 through *Agrobacterium*-mediated transfer. Transformants were successfully identified via GUS testing and PCR analysis. The antibacterial activity of Pen3a duckweed against *E. coli* was measured to be approximately 19.2 ± 0.6 mm, while it was around 15.5 ± 0.5 mm against *S. aureus*, as observed in inhibition zone assays (Yang et al. 2023). Furthermore, the expression of genes associated with sphingolipid metabolism and the phagocytosis process was found to be up-regulated in Pen3a-expressing duckweed (Yang et al. 2023). These findings underscore the potential of Penaeidins 3a as promising candidates for enhancing disease resistance in agricultural and aquacultural settings, highlighting the importance of further research in this field.

Duckweeds as edible vaccine platform for porcine epidemic diarrhoea

Porcine epidemic diarrhea (PED) stands as a prevalent affliction in swine, particularly lethal to newborn piglets, posing substantial challenges to swine farming productivity and sustainability (Yu et al. 2024). The causative agent, Porcine Epidemic Diarrhea Virus (PEDV), is an RNA virus classified within the *Alphacoronavirus* genus of the *Coronaviridae* family (Turlewicz-Podbielska and Pomorska-Mól, 2021). Among its distinctive features, PEDV harbors a spike protein (PEDV-SpikeI) crucial for viral entry into host cells, thus representing an attractive target for vaccine development against PEDV (Li et al. 2020). Similar to other coronaviruses, PEDV-SpikeI facilitates interaction with host cell receptors, initiating infection processes. Consequently, targeting this protein in vaccine formulations holds promise for mitigating PEDV outbreaks and minimizing associated economic losses within the swine industry. Continuing research efforts aimed at elucidating PEDV pathogenesis and refining vaccine strategies are imperative for effective disease management and safeguarding swine health and welfare.

In a groundbreaking study by Ko et al. (2011), *L. minor* fronds underwent transformation using *A. tumefaciens* to incorporate a gene encoding PEDV-SpikeI fused with a c-myc tag, facilitating subsequent protein purification processes. Following transformation, the resultant fronds underwent rigorous antibiotic screening, with successful transformants subsequently subcultured to initiate vegetative growth. Comprehensive analyses utilizing PCR, RT-PCR, and Western blot techniques yielded confirmatory outcomes: PCR analysis revealed the presence of a distinct 330-bp band corresponding to the primer for the PEDV-SpikeI gene, while RT-PCR results validated successful transcription of the inserted gene within the fronds (Ko et al. 2011). Furthermore, Western blot analysis corroborated these findings by demonstrating the presence of a 28-kDa protein, consistent with the anticipated size of the PEDV-SpikeI-myc fusion protein (Ko et al. 2011). This pioneering research showcases the potential of duckweed as a versatile platform for heterologous protein expression, laying the groundwork for further advancements in vaccine development against PEDV and other viral pathogens.

Duckweeds as edible vaccine for haemorrhagic disease

Grass Carp Haemorrhagic Disease (GCHD) stands as a prevalent affliction in grass carp fish, with staggering losses in rearing estimated to reach up to 85% due to its impact (Liang et al. 2014). The causative agent, Grass Carp Reovirus (GCRV), a double-stranded RNA virus classified within the genus *Aquareovirus* of the *Reoviridae* family, serves as the primary culprit behind GCHD (He et al. 2017). Among the key antigen candidates investigated for GCRV, the VP35 protein, encoded by the S11 gene, has emerged as a focal point due to its pivotal role in GCRV pathogenesis (Mu et al. 2020; Zeng et al. 2021). Notably, the VP35 protein harbors a conserved putative zinc-binding motif CxxC-n16-HxC sequence, adding to its significance (Gao et al. 2018). In a significant breakthrough, Zhu et al. (2022) succeeded in transforming *A. tumefaciens* to carry the S11 gene utilizing the pCAMBIA1303 plasmid. This transformed *Agrobacterium* strain was then introduced to the callus of *L. aequinoctialis*, induced through the application of 2,4-dichlorophenoxyacetic acid (2,4-D) and thidiazuron (TDZ), serving as a pivotal step in the development of a potential vaccine against GCHD (Zhu et al. 2022). Such advancements underscore the promise of duckweed as a versatile platform for heterologous protein expression, opening new avenues for combating devastating diseases in aquatic ecosystems.

After the successful introduction of the S11 gene into the callus of *L. aequinoctialis*, select transformants underwent further cultivation to regenerate into fronds (Zhu et al. 2022). The confirmation of transformation efficacy was conducted through a series of assays. GUS assays revealed distinct blue coloration in both roots and leaves of the transformed fronds, contrasting with the absence of color change in non-transformed fronds. PCR analysis provided conclusive evidence of S11 gene integration into the genomic DNA of *L. aequinoctialis*. Western blot analysis corroborated these findings by detecting a prominent 50 kDa immunoreactive band, indicative of the presence of the VP35 protein (Zhu et al. 2022). Additionally, fluorescence observations unveiled a conspicuous green fluorescent signal in the transformant fronds, further validating the successful expression of the introduced gene (Zhu et al. 2022). This comprehensive validation underscores the robustness of the transformation process and the potential of the engineered duckweed as a promising platform for future research in disease management.

Duckweeds as edible vaccine for vibriosis

Vibriosis poses a significant threat to aquatic animal farming, affecting a wide range of species such as fish, shrimp, prawn, clam, and crab (Arunkumar et al. 2020). Among the various types of aquatic animals susceptible to vibriosis are Barramundi Perch (*Lates calcarifer*), Summer Flounder (*Paralichthys dentatus*), Malabar Grouper (*Epinephelus malabaricus*), Pacific white shrimp (*Litopenaeus vannamei*), red prawn (*Solenocera sub-uda*), Indian prawn (*Fenneropenaeus indicus*), carpet clam (*Paphia textile*), and mud crab (*Scylla serrata*) (Ina-Salwany et al. 2019). Vibriosis is primarily caused by gram-negative bacteria belonging to the *Vibrio* genus, including *V. harveyi*, *V. anguillarum*, *V. alginolyticus*, and *V. parahaemolyticus* (Zhang et al. 2022). These bacteria commonly infect various internal organs such as the gills, skin, intestinal tract, kidney, and liver of the host organisms (Chin et al. 2020). Traditional methods for preventing vibriosis often involve incorporating prebiotics into the feed. These prebiotic mixtures typically comprise yeast, microalgae, gram-positive bacteria, and gram-negative bacteria (Elias et al. 2023). Incorporating prebiotics into feed has been demonstrated to stimulate the production of antagonistic compounds and organic acids, regulate immune responses, and enhance feed conversion efficiency (Krysiak et al. 2021). Such interventions aim to bolster the overall health and resilience of aquatic animals, reducing the incidence and severity of vibriosis outbreaks in aquaculture settings.

Vaccination offers a promising strategy for more targeted and effective prevention against vibriosis compared to traditional methods like prebiotic supplementation. Vaccines can stimulate the development of specific immune responses tailored to combat *Vibrio* spp. infections (Kulkarni et al. 2021). One avenue of vaccine development targets *Vibrio* outer membrane proteins (OMPs), which exhibit high conservation across the *Vibrio* genus (Goh et al. 2022). Among these OMPs, the LamB protein has garnered attention as a potential vaccine antigen (Lun et al. 2014). To develop an edible vaccine based on LamB, the LamB gene was incorporated into the pMYC plasmid and introduced into disarmed *A. tumefaciens* EHA105 using a heat shock method (Heenatigala et al. 2020). The transformed agrobacteria were then mixed with *W. arrhiza* biomass, and transformants were selected using hygromycin. This process allows for the expression of LamB within the duckweed biomass, offering a convenient and potentially cost-effective means of administering vaccines to aquatic organisms susceptible to vibriosis (Heenatigala et al. 2020). Such vaccines hold promise for enhancing the immune defenses of aquatic animals and reducing the incidence and severity of vibriosis outbreaks in aquaculture settings.

The success of the transformation process was confirmed through multiple analyses, including PCR, RT-PCR, and immunoblotting (Heenatigala et al. 2020). PCR and RT-PCR results provided evidence of the integration and heterologous expression of the LamB gene within the plant genome. Immunoblotting further corroborated these findings by detecting a distinct band corresponding to the recombinant LamB protein, measuring approximately 52 kDa. Subsequent clinical trials involved feeding zebrafish (*Danio rerio* AB strain) challenged with *V. alginolyticus*, a bacterium responsible for vibriosis (Heenatigala et al. 2020). Remarkably, fish fed a diet comprising half normal feed and half transgenic *W. arrhiza* exhibited a mortality rate of only 36.7% when infected with vibriosis. In stark contrast, the mortality rate was 100% among fish fed only a normal diet, with all individuals succumbing to the infection after exposure to *V. alginolyticus* (Heenatigala et al. 2020). These findings underscore the efficacy of edible vaccines in enhancing the survival of fish afflicted with vibriosis, offering a promising avenue for disease management in aquatic ecosystems.

Future directions for the development of edible vaccines from duckweeds

In the animal farming industry, edible vaccines represent a paradigm shift in disease prevention strategies, offering a unique combination of immunization and nutrition in a single delivery system. The development of edible vaccines is highly incentivized, as they could streamline the vaccination process by delivering vaccines while simultaneously providing nutrients (Du et al. 2022). This dual functionality not only simplifies vaccination protocols but also addresses nutritional needs, promoting overall health and well-being in livestock populations. However, edible vaccines are still a nascent concept compared to well-established injectable vaccines. It is evident that more proof-of-concept studies are required to bring edible vaccines closer to the current standards of conventional vaccines. Additionally, as discussed above, duckweeds, and plant expression systems in general, still have several drawbacks that might impede the development of plant-based edible vaccines. The most prevalent of these are limited growth rates and yield. Most of these impediments stem from the underdevelopment of the plant expression system, which is exacerbated by the preference for bacterial and mammalian expression systems. This preference has led to greater research efforts focused on their development, creating a cycle that has overshadowed the plant expression system despite its many advantages and potential.

There are two major areas for future development concerning the use of duckweeds as an edible vaccine platform: the development of edible vaccines using duckweeds and the improvement of duckweeds as a platform for heterologous protein expression. In the first area, a key focus is the stability and storage of duckweed-based edible vaccines. This involves investigating the shelf life of the vaccines and developing methods to enhance their stability during storage and handling (Kumar et al. 2022). Effective post-harvest techniques will be necessary to maintain vaccine efficacy and facilitate ease of use and distribution. Another focus is the economic viability of duckweed-based edible vaccines, especially in comparison to conventional ones. This includes assessing the costs associated with cultivation, harvesting, and processing. Developing strategies for scaling up production will be essential to meet potential commercial demands and ensure the practicality of using duckweeds as a vaccine platform. Establishing clear regulatory pathways for plant-based edible vaccines will also be crucial for their approval and widespread use (Parvathy 2020). Research should focus on developing guidelines that ensure compliance with safety standards and efficacy requirements. Additionally, comprehensive safety assessments are necessary to evaluate any potential toxicity or allergenicity of the vaccine products (EFSA Panel on Genetically Modified Organisms (GMO) et al. 2022).

In the second area, a critical aspect for future development is optimizing the duckweed expression system to maximize yield, functionality, effectiveness, and safety (Trombetta et al. 2022). Harnessing the potential of duckweed-based edible vaccines will require enhancing genetic engineering techniques to optimize the production of vaccine antigens in duckweeds. This includes refining gene constructs to improve antigen expression, selecting suitable promoters, and utilizing advanced transformation methods for efficient integration of vaccine genes into duckweed genomes (Lee et al. 2023). Exploring innovative genetic modifications could further enhance the effectiveness of the vaccine production process. Additionally, studying how duckweeds manage PTMs will be crucial for ensuring that vaccine proteins are properly folded and functional (Lee et al. 2023). To validate the potential of duckweed-based vaccines, extensive preclinical studies using animal models are essential. These studies should assess the immunogenicity, efficacy, and safety of the vaccines, including determining the optimal dosage and delivery methods (Naasani 2022). Ensuring that the vaccines elicit strong and long-lasting immune responses will be key to their success.

Conclusions

The development of duckweeds as edible vaccines represents a strategic approach to harnessing the full potential of these aquatic plants. Renowned for their versatility, duckweeds have long served as valuable sources of nutrition for various animal species. Their widespread adoption as animal feed stems from their rapid growth rates, ease of cultivation, and nutritional richness. By incorporating duckweeds into animal diets, farmers can not only ensure adequate nutrition for their livestock but also mitigate feed procurement costs. The concept of edible vaccines involves engineering duckweeds to express antigens or immunogenic proteins that, when consumed by animals, stimulate an immune response, thereby providing protection against specific diseases. This approach offers several advantages, including ease of administration, reduced stress on animals, and potentially enhanced vaccine efficacy through mucosal immune stimulation. As research progresses, duckweeds are being genetically engineered to express a diverse array of vaccine antigens targeting various infectious diseases prevalent in livestock and aquaculture. By leveraging the remarkable attributes of duckweeds, scientists aim to revolutionize the field of veterinary medicine by providing a cost-effective, sustainable, and efficient means of disease prevention in animal populations. Through continued innovation and refinement, duckweed-based edible vaccines hold immense promise for safeguarding animal health and welfare in agricultural and aquaculture settings.

Moreover, duckweeds possess inherent capabilities for producing heterologous proteins, making them attractive candidates for vaccine development. Studies have demonstrated that duckweeds can generate heterologous proteins with titers comparable to those achieved in more established plant models. By leveraging these natural protein production capabilities, researchers have embarked on extensive efforts to utilize duckweeds as a versatile platform for edible vaccine production. However, challenges regarding the development of duckweed-based edible vaccines persist. These include the novelty of edible vaccines, which still presents significant gaps between edible and injectable vaccines. Inherent limitations of the plant expression system are also significant obstacles, stemming from the underdevelopment of the platform compared to more conventional systems. Nevertheless, research is ongoing to improve these conditions and maximize the potential of duckweeds as an edible vaccine platform.

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Declarations

Conflict of interest The authors affirm that there are no conflicts of interest, financial or personal, that could have influenced the research presented in this paper.

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