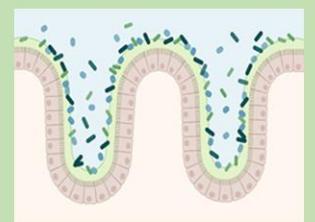


## WP1.1 – Microalgae as feed additives

### Study of the Current State of Knowledge and Market Research

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# 1 SUMMARY

## English summary

This study provides a critical assessment of the current state of knowledge on the use of microalgae in animal feed, with a particular focus on three closely interconnected domains: gut health, immune function, and modulation of the intestinal microbiome. Special attention is given to monogastric animals, especially pigs and poultry, where intestinal dysfunctions, post-weaning stress, and immune imbalance represent major challenges for animal health and productivity.

The analysis of experimental and review studies indicates that microalgae—most notably *Chlorella vulgaris* and *Arthrospira platensis*—may beneficially affect intestinal morphology, gut environment stability, and microbial composition when applied at appropriate inclusion levels and in suitable forms. These effects are frequently interpreted as prebiotic or gut barrier-supporting; however, direct functional assessments of intestinal barrier integrity and clear causal links to immune responses are often lacking.

Immunomodulatory effects of microalgae are commonly reported in the literature, yet the supporting evidence remains limited and is largely based on indirect markers such as antioxidant status or changes in circulating immune parameters.

The most consistent and methodologically robust evidence relates to the capacity of microalgae to modulate microbial communities within the digestive tract, particularly in ruminants, where *in vivo* studies demonstrate measurable effects on rumen microbiota and fermentation patterns. In monogastric animals, microbiome-related evidence is more limited and frequently descriptive, with insufficient functional interpretation.

In addition, the study reviews the EU regulatory framework and relevant national legislation in the Czech Republic and Austria, summarizes key European research projects, patent activities, and commercially available products. Based on an integrated critical synthesis, the study identifies major research gaps and formulates recommendations for future research and application of microalgae as functional feed additives targeting gut health and microbiome modulation.

## Český souhrn

Tato studie poskytuje kritické zhodnocení současného stavu poznání o využití mikrořas v krmivech hospodářských zvířat se zaměřením na tři vzájemně propojené oblasti: zdraví střeva, imunitní odpověď a modulaci střevního mikrobiomu. Pozornost je věnována především monogastrickým zvířatům, zejména prasatům a drůbeži, u nichž jsou střevní dysbalance, post-odstavový stres a poruchy imunity významnými faktory ovlivňujícími zdraví i produkční výsledky.

Analýza dostupných experimentálních a přehledových studií ukazuje, že mikrořasy – zejména druhy *Chlorella vulgaris* a *Arthrospira platensis* – mohou při vhodném dávkování a formě zpracování příznivě ovlivňovat morfologii střevní sliznice, stabilitu střevního prostředí a složení mikrobiální komunity. Tyto účinky jsou často interpretovány jako prebiotické či podpůrné pro střevní bariéru, avšak ve většině studií chybí přímé funkční hodnocení integrity střevní bariéry a kauzální propojení s imunitní odpovědí.

Imunomodulační účinky mikrořas jsou v literatuře často deklarovány, avšak jejich experimentální opora je omezená a převážně založená na nepřímých ukazatelích, jako jsou antioxidační markery nebo změny cirkulujících imunologických parametrů. Přímá data o lokální střevní imunitě a funkčních imunitních odpovědích jsou vzácná.

Nejkonzistentnější důkazy se týkají schopnosti mikrořas modulovat mikrobiální komunity v trávicím traktu, zejména u přežvýkavců, kde *in vivo* studie dokumentují změny rumen mikrobiomu a fermentačních procesů. U monogastrických zvířat jsou data omezenější a často postrádají funkční interpretaci mikrobiomových změn.

Studie dále shrnuje regulatorní rámec EU a národní legislativu v ČR a Rakousku, analyzuje relevantní evropské výzkumné projekty, patentovou aktivitu a dostupné produkty na trhu. Na základě kritické syntézy identifikuje klíčové výzkumné mezery a formuluje doporučení pro budoucí výzkum a aplikační využití mikrořas jako funkčních doplňků krmiv se zaměřením na zdraví střeva a mikrobiom.

### **Německý souhrn**

Diese Studie bietet eine kritische Bewertung des aktuellen Wissensstands zur Nutzung von Mikroalgen in Futtermitteln für Nutztiere mit Schwerpunkt auf drei eng miteinander verknüpften Bereichen: Darmgesundheit, Immunfunktion und Modulation des intestinalen Mikrobioms. Der Fokus liegt insbesondere auf monogastrischen Tieren, vor allem Schweinen und Geflügel, bei denen Darmstörungen, Absetzstress und immunologische Dysbalancen wesentliche Herausforderungen für Tiergesundheit und Leistung darstellen.

Die Analyse experimenteller und übersichtsartiger Studien zeigt, dass Mikroalgen – insbesondere *Chlorella vulgaris* und *Arthrospira platensis* – bei geeigneter Dosierung und Verarbeitung positive Effekte auf die Morphologie der Darmschleimhaut, die Stabilität des intestinalen Milieus und die Zusammensetzung der mikrobiellen Gemeinschaft haben können. Diese Effekte werden häufig als präbiotisch oder als Unterstützung der Darmbarriere interpretiert; jedoch fehlen in vielen Studien direkte funktionelle Messungen der Barrierefintegrität sowie klare kausale Zusammenhänge zur Immunantwort.

Immunmodulatorische Wirkungen von Mikroalgen werden in der Literatur häufig beschrieben, sind jedoch überwiegend durch indirekte Marker wie antioxidative Parameter oder Veränderungen zirkulierender Immunmarker belegt.

Die konsistentesten und methodisch belastbarsten Ergebnisse betreffen die Modulation mikrobieller Gemeinschaften im Verdauungstrakt, insbesondere bei Wiederkäuern, wo *In-vivo*-Studien Veränderungen des Pansenmikrobioms und der Fermentationsprozesse dokumentieren. Bei monogastrischen Tieren sind die verfügbaren Daten zum intestinalen Mikrobiom eingeschränkter und häufig rein deskriptiv.

Darüber hinaus behandelt die Studie den regulatorischen Rahmen der EU sowie die nationale Gesetzgebung in Tschechien und Österreich, fasst relevante europäische Forschungsprojekte, Patentaktivitäten und Marktprodukte zusammen und identifiziert zentrale Forschungslücken. Auf dieser Basis werden Empfehlungen für zukünftige Forschung und die praxisnahe Anwendung von Mikroalgen als funktionelle Futtermittelzusätze mit Fokus auf Darmgesundheit und Mikrobiom formuliert.

## 2 USE MICROALGAE IN FEED ADDITIVES

Microalgae represent a promising group of feed resources for livestock nutrition, both in terms of their nutritional value and their content of biologically active compounds with potential effects on animal health. Interest in their use in animal feeds has increased significantly in recent years, particularly in response to pressure to reduce the use of antibiotics as growth promoters, efforts to improve gut health and immune function, and the search for more sustainable alternatives to conventional feed ingredients such as soybean meal or fishmeal.

Microalgae are unicellular or simple multicellular photosynthetic organisms characterised by high biochemical diversity. Depending on the species and cultivation conditions, they may contain high levels of high-quality proteins, essential amino acids, lipids including polyunsaturated fatty acids (especially n-3 PUFA), vitamins, minerals, carotenoids, phenolic compounds, and structural polysaccharides such as  $\beta$ -glucans or other non-digestible carbohydrates with potential prebiotic effects.

In the context of monogastric animal nutrition, particularly in poultry and pigs, attention is primarily focused on the ability of microalgae to positively influence gut health. After weaning or under intensive production conditions, these animals frequently experience disruption of the intestinal barrier, alterations in microbial balance, and impairment of immune function. A number of studies indicate that low to moderate inclusion levels of microalgae, especially species such as *Chlorella vulgaris* and *Arthrospira platensis* (*Spirulina*), can support intestinal mucosal development, increase villus height, affect the villus-to-crypt ratio, and modulate the composition of the intestinal microbiota towards a higher abundance of potentially beneficial bacteria, such as lactobacilli.

An important role is played by both structural polysaccharides of the microalgal cell wall, which may act as prebiotics, and bioactive compounds with immunomodulatory, antioxidant, and anti-inflammatory properties. These characteristics are considered particularly relevant during periods when animals are exposed to stress factors such as weaning, high stocking density, or heat stress.

In ruminants, the use of microalgae has been studied mainly in relation to their effects on rumen fermentation, nutrient metabolism, and the fatty acid composition of animal-derived products. Certain microalgae, particularly oleaginous species such as *Schizochytrium limacinum*, have been investigated as sources of energy and n-3 fatty acids with the potential to influence ruminal fermentation patterns, volatile fatty acid production, and animal energy metabolism, for example during periods of heat stress. At the same time, several studies have reported changes in rumen microbial composition, including effects on methanogenic microorganisms, highlighting the need for careful evaluation of the microalgal species, dosage, and target animal category.

From the perspective of current research, it is evident that the effects of microalgae in animal feeds are strongly dependent on the microalgal species used, its biochemical composition, the form of processing (whole biomass, defatted biomass, extracts), the inclusion level, and the physiological status and species of the animal. Although an increasing number of experimental studies are available, many results remain difficult to compare and some effects are still ambiguous, particularly with respect to long-term impacts and the mechanisms underlying interactions between microalgae, the gut microbiota, and the immune system.

This study therefore focuses on a systematic evaluation of available knowledge on the use of microalgae in livestock feeds, with particular emphasis on their potential to improve gut health, modulate the intestinal microbiome, and support immune functions. Priority is given to microalgal species that are already being studied or applied in practice, especially in feeds for poultry and pigs,

and whose effects are relevant for the development of alternatives to antibiotic growth promoters under modern livestock production conditions.

### 3 LEGISLATION FRAMEWORK

Microalgae represent a promising and rapidly developing component in the field of animal nutrition. Thanks to their high content of proteins, essential fatty acids, pigments, antioxidants, and other bioactive compounds, their use in feed is emerging as a nutritionally valuable alternative to traditional raw materials. In recent years, microalgae have been applied not only in feeds for fish in aquaculture and for livestock, but also in pet nutrition. With the growing interest in sustainable and innovative approaches to animal production, the share of microalgae in feeds is expected to continue increasing. An undeniable advantage is that, unlike “Novel Foods,” the use of microalgae in feed is not limited only to approved species. Nevertheless, their use is not without a legislative framework.

In order to use microalgae or products derived from them in feed, a number of legal requirements must be met. The basic framework is laid down by Regulation (EC) **No 178/2002** of the European Parliament and of the Council, as amended, which establishes the general principles and requirements of food law, sets up the European Food Safety Authority, and lays down procedures in matters of food safety. Although it is often mentioned in connection with food for human consumption, it also applies to feed, as it establishes fundamental obligations and principles of safety, traceability, responsibility, and crisis management across the entire food and feed chain. Any feed placed on the EU market must be safe, must not endanger human or animal health, and its origin must be traceable.

Specific rules for placing feed on the market are laid down in Regulation (EC) **No 767/2009** of the European Parliament and of the Council, as amended, which, among other things, distinguishes between feed materials and feed additives. This distinction is also crucial for the use of microalgae.

Feed materials primarily serve as a nutritional component (e.g. a source of proteins or fatty acids). If a microalga fulfils this role, it may be placed on the market as a feed material provided that it is listed in the EU Catalogue of Feed Materials, established by Commission Regulation (EU) **No 68/2013**.

Microalgae are included in Part C – List of Feed Materials, Section 7 – Other plants, algae, fungi and products derived thereof. Although the use of the Catalogue is voluntary, in practice it serves as an officially recognised reference framework and helps with the labelling and control of feed products.

If a producer or manufacturer places a new feed material on the market that is not listed in the Catalogue, they are obliged to register this material in the Feed Materials Register. The Register is managed by the European feed industry and serves to ensure transparency and a harmonised approach. The obligation to register applies, for example, to new species of microalgae, extracts, or technologically modified products that are not explicitly listed in the Catalogue. The manufacturer is therefore obliged to first verify whether the given material is included in the Catalogue. If it is not, it cannot be legally placed on the market without registration.

A feed additive is defined under Regulation (EC) **No 1831/2003** of the European Parliament and of the Council as a substance, microorganism, or preparation that is not a feed material and is intentionally added to feed to fulfil a specific function – for example a technological, sensory, nutritional, or health-related function. Typical examples in the case of microalgae include natural colourants (e.g. astaxanthin), antioxidants, substances supporting digestion, enhancing immune functions, or influencing product quality (colour of meat, eggs, etc.).

If a microalga or a substance derived from it therefore serves a purpose other than mere nutrition, it must be authorised by the European Food Safety Authority (EFSA). The assessment focuses on safety

for target animals, consumers, workers, and the environment, as well as on the efficacy of the declared function. Based on EFSA's opinion, the European Commission decides on the authorisation of the additive. Authorised additives are subsequently included in the official EU Register of Feed Additives.

In the Czech Republic, the use of feed and its placing on the market is also governed by national legislation, in particular Act No. **91/1996** Coll., on feed, as amended. The performance of public administration and supervision in this area is ensured by the Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ), which provides basic information and links to European legislation on its website.

Austria, feed legislation is governed by the same core European regulations as in the Czech Republic, in particular the rules on placing feed on the market and the safety requirements laid down in general EU legislation (e.g. Regulation (EC) No **767/2009** or Regulation (EC) No **178/2002** of the European Parliament and of the Council). Austrian national law complements these provisions through the **Futtermittelgesetz 1999** (BGBI. I Nr. **139/1999**) and the related **Futtermittelverordnung 2010** (BGBI. II Nr. **316/2010**), which regulate the responsibilities of authorities, the rights and obligations of operators, and possible national derogations or sanctions for breaches of feed legislation. According to the Austrian authorities (Federal Office for Food Safety, BAES), feed may be placed on the market and used only if it complies with both EU regulations and Austrian legislation and is safe for animals, humans, and the environment. For manufacturers, this entails an obligation to register the establishment or production activity with BAES and to comply with feed hygiene standards (e.g. HACCP), including regular inspections of both raw materials and final products. Austria also carries out national feed quality controls, for example with regard to unauthorised GMO substances, with inspection intensity adjusted to the risk profile of individual commodities. Although Austrian legislation does not contain specific provisions exclusively addressing microalgae, their use in feed is subject to the same general obligations: they must meet European safety and registration requirements according to the nature of their use (e.g. as a feed material or an additive), and operators must comply with these requirements when placing them on the market.

## 4 RESEARCH AND DEVELOPMENT

### 4.1 Introduction

The use of microalgae in feeds for livestock has attracted markedly increased interest over the past two decades, driven both by technological advances in microalgae cultivation and processing and by broader changes in livestock production systems. The gradual withdrawal from routine use of antibiotic growth promoters, increasing pressure for more sustainable production, and a growing emphasis on animal welfare have led to the search for alternative feeding strategies that support animal health through natural mechanisms. In this context, microalgae are increasingly perceived not only as sources of high-quality nutrients but also as potential functional feed components with targeted effects on gut health, immune responses, and the composition of the intestinal microbiome (Abd El-Hack et al., 2022; Van Nerom et al., 2024).

From an animal nutrition perspective, it is essential to clearly distinguish between two fundamental roles of microalgae in animal diets. On the one hand, microalgae may function as a feed ingredient, serving as a source of protein, lipids, vitamins, or minerals, often included at relatively high levels in the ration. On the other hand, microalgae or their fractions may be used as feed additives, where a biologically specific effect is expected at low inclusion levels, for example through modulation of the gut environment, the intestinal microbiome, or immune responses. This distinction is crucial for the interpretation of experimental results, as many studies attribute positive “health” effects to microalgae that may, in fact, result from a general improvement in the nutritional quality of the diet rather than from a specific bioactivity of microalgae as functional components.

In recent years, particular attention has been devoted to the relationship between nutrition, gut health, and the immune system, with the intestinal microbiome recognised as a key mediator of these interactions. The gut represents not only the primary site of digestion and nutrient absorption but also the largest immune organ in the body, whose function is closely linked to the composition and activity of the resident microbial community. Any dietary intervention that affects the structure of the intestinal microbiome, the production of microbial metabolites (e.g. short-chain fatty acids), or the integrity of the intestinal barrier may therefore have direct consequences for both local and systemic immune responses. Owing to their complex chemical composition, including structural cell wall polysaccharides, bioactive lipids (particularly n-3 polyunsaturated fatty acids), pigments, and antioxidants, microalgae constitute a particularly interesting group of feed resources in this context.

However, it should be emphasised that the available scientific literature is characterised by considerable heterogeneity in terms of microalgal species used, experimental designs, inclusion levels, biomass processing methods, and evaluated parameters. While studies in monogastric animals, especially poultry and pigs, often directly assess gut health indicators, intestinal morphology, or the composition of the intestinal microbiota (e.g. Pestana et al., 2022; Ahmed et al., 2025), research in ruminants has primarily focused on rumen fermentation, lipid metabolism, and production traits. Consequently, extrapolating conclusions from these studies to the concept of “gut health” in the narrower sense requires cautious and critical interpretation.

The objective of this overview is not to provide a scientific review, but rather to present a structured synthesis of published research on the use of microalgae in feeds for livestock, with a focus on three closely interconnected areas: (i) gut health, (ii) immune system support, and (iii) modulation of the intestinal microbiome. The text aims to identify effects of microalgae that are genuinely supported by experimental evidence, to analyse methodological limitations of existing studies, and to formulate

recommendations for future research, particularly with regard to the practical applicability of microalgae as functional feed additives.

## 4.2 Effect of Microalgae on Gut Health in Livestock

Gut health represents one of the key determinants of overall health status and productive performance in livestock. Functional integrity of the intestinal mucosa, a balanced microbial community, and an adequate local immune response are essential for efficient nutrient utilisation, prevention of enteric diseases, and maintenance of metabolic homeostasis. Consequently, animal nutrition research has increasingly focused on dietary interventions that specifically support gut health, with microalgae being investigated in this context as potential functional feed components.

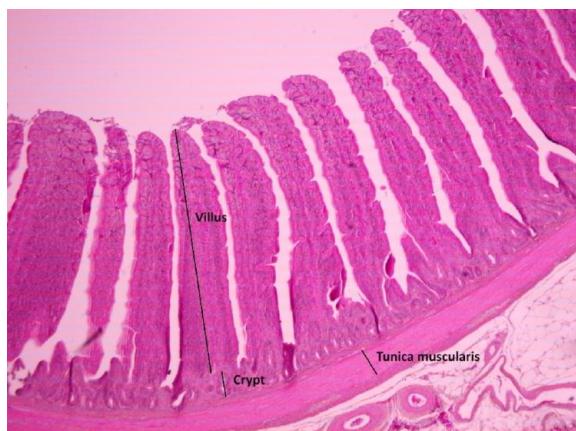
The mechanisms by which microalgae may influence gut health are complex and multifactorial. They include both direct effects of specific microalgal components on the intestinal epithelium and indirect effects mediated through changes in the composition and activity of the gut microbiome. The cell walls of many microalgal species contain structural polysaccharides that are poorly digestible for the host but may serve as substrates for intestinal microorganisms. These polysaccharides may therefore exert prebiotic effects, similar to certain dietary fibre fractions, and contribute to increased production of short-chain fatty acids, particularly acetate, propionate, and butyrate, which are considered key metabolites supporting intestinal barrier integrity.

A substantial proportion of available experimental evidence originates from studies conducted in monogastric animals, particularly pigs and poultry, where gut health parameters can be assessed relatively directly. A study on weaned piglets evaluating dietary supplementation with the microalga *Chlorella vulgaris* provides one of the few examples of research that systematically links growth performance, nutrient digestibility, intestinal morphology, and gut microbiota composition (Pestana et al., 2022). The authors report that inclusion of *C. vulgaris* in the diet did not impair growth performance but had measurable effects on certain aspects of the intestinal environment. At the same time, they highlight the limited bioavailability of nutrients bound within the rigid microalgal cell wall and the potential need for exogenous enzymes to achieve the full nutritional and functional potential of the biomass.

This aspect is critical for interpreting the effects of microalgae on gut health. When microalgae are administered as whole, intact biomass, the rigid cell wall may restrict the release of bioactive compounds in the upper sections of the gastrointestinal tract and shift their activity towards more distal intestinal segments, where microbial enzymes are available. Such a shift may be desirable from the perspective of microbiome modulation but simultaneously complicates the distinction between direct effects of microalgae on the epithelium and indirect effects mediated by microbial activity. In this respect, studies combining microalgae with enzyme preparations represent an important step towards elucidating mechanisms of action; however, they also raise the question of whether the observed effects result from the microalgae themselves or from synergistic interactions among multiple dietary components.

In **poultry**, gut health has long been regarded as a key factor influencing not only growth performance and feed conversion but also resistance to enteric pathogens and overall production stability. Several studies explicitly frame microalgae as potential alternatives to conventional prebiotics or antibiotic growth promoters. Research investigating the use of *Chlorella vulgaris* as a prebiotic alternative in broiler chickens interprets observed changes in performance and health status primarily in the context of improvements in the intestinal environment (Ahmed et al., 2025). Although detailed mechanistic data on intestinal barrier integrity or permeability are not always

available in such studies, the conceptual shift of microalgae from the role of a “nutritional ingredient” to that of a “functional additive” is of major importance for future research.



Microscopic image of a hematoxylin and eosin staining of a histological section of the duodenum of broilers. Villus height, crypt depth and thickness of the tunica muscularis are indicated. (S. Van Nerom et al., 2024)

Review papers focusing on poultry further suggest that microalgae may positively affect intestinal mucosal morphology, particularly parameters such as villus height, crypt depth, and the villus-to-crypt ratio, which are commonly used as indicators of intestinal absorptive capacity (Abd El-Hack et al., 2022; Van Nerom et al., 2024). These changes are typically interpreted as evidence of improved gut health; however, it should be noted that morphological parameters alone do not necessarily reflect functional barrier integrity. Improvements in epithelial structure may result from generally enhanced nutritional supply rather than from specific bioactive effects of microalgae.

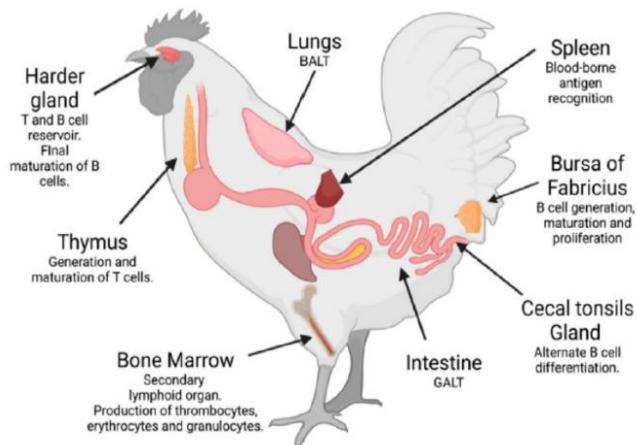
In **ruminants**, the situation differs substantially, as the primary fermentation processes occur in the rumen, which represents a unique microbial ecosystem with limited analogy to the intestinal tract of monogastric animals. Nevertheless, some studies may indirectly contribute to understanding the relationship between microalgae and “digestive tract health” in a broader sense. In vitro studies on rumen fermentation demonstrate that different microalgal species can influence volatile fatty acid production, fermentation pH, and gas or methane output (Sucu, 2020). These changes may have implications for fermentation stability and the prevention of metabolic disorders such as subacute ruminal acidosis; however, their direct relevance to intestinal mucosal health is limited.

In vivo studies in ruminants that assess changes in rumen microbiota and fermentation parameters following microalgae supplementation provide valuable insights into ecological interactions between dietary components and microbial communities. However, these studies rarely evaluate parameters related to epithelial integrity or inflammatory responses in post-ruminal sections of the gastrointestinal tract. Consequently, when discussing “gut health” in ruminants, it is essential to clearly define whether the term refers to rumen fermentation stability or to actual health of the intestinal mucosa in distal segments.

Overall, the available literature provides some indications of potentially positive effects of microalgae on gut health, particularly in monogastric animals. However, these indications are often based on indirect markers or a limited set of evaluated parameters. Systematic studies simultaneously assessing intestinal morphology, functional barrier integrity, microbial metabolite production, and clinical indicators of gut health remain largely lacking. Without such a comprehensive approach, it remains difficult to conclusively determine whether the observed effects of microalgae represent a genuine functional benefit for gut health or merely a secondary consequence of changes in diet composition and nutritional value.

### 4.3 Immunomodulatory Effects of Microalgae in Livestock Feeds

The immune system of livestock is closely linked to the condition of the gastrointestinal tract and is strongly influenced by nutrition, particularly during periods of increased stress such as weaning, dietary transitions, heat stress, or high production load. In this context, microalgae are frequently cited in the literature as potential immunomodulatory feed components capable of influencing both innate and adaptive immune responses. However, it is essential to critically assess to what extent these effects are genuinely supported by experimental data and to what extent they are inferred from indirect indicators or generalised mechanistic assumptions.



Immune system of chickens: primary lymphoid organs bursa of Fabricius and thymus. Secondary lymphoid organs spleen and bone marrow. (S. Van Nerom et al., 2024)

From a biological perspective, the potential immunomodulatory effects of microalgae may be explained by several parallel mechanisms. Microalgae contain a wide range of bioactive compounds, including polyunsaturated fatty acids, particularly of the n-3 series (e.g. DHA in the genus *Schizochytrium*), pigments and antioxidants (phycocyanin in *Spirulina/Arthrospira*, carotenoids), vitamins, and structural cell wall polysaccharides. These components may act either directly on host immune cells or indirectly via alterations in the gut microbiome and the production of microbial

metabolites that modulate the immune “set-point” of mucosal immunity.

Within the available body of publications, direct experimental data focusing on immune parameters remain relatively limited and are often embedded within broader nutritional studies. The most comprehensive overviews of immunomodulatory effects of microalgae are provided by review papers focused on poultry, which summarise results from multiple *in vivo* studies using different microalgal species (Abd El-Hack et al., 2022; Van Nerom et al., 2024). These reviews report that microalgae supplementation may be associated with changes in both innate and adaptive immune parameters, including alterations in circulating immunoglobulin levels, changes in cytokine expression, or relative changes in the weight of immune organs such as the bursa of Fabricius, thymus, or spleen.

However, interpretation of these findings is often complicated by several factors. First, immunological parameters are frequently measured only in serum or plasma, without assessment of local immune responses in the intestinal mucosa, which are critical for understanding the relationship between nutrition, the microbiome, and immunity. Second, changes in immune markers are not always linked to functional outcomes, such as increased resistance to infection or improved vaccination responses. In such cases, it cannot be unequivocally concluded that the observed changes represent true “immune enhancement” rather than mere modulation of selected laboratory indicators.

A common feature of studies evaluating the immunomodulatory effects of microalgae is the use of antioxidant markers as indirect evidence of improved immune function. Reductions in malondialdehyde (MDA) concentrations or increases in the activity of antioxidant defence enzymes, such as superoxide dismutase (SOD) or glutathione peroxidase (GPx), are frequently interpreted as

indicators of improved immune status. Although oxidative stress and inflammatory responses are closely interconnected, these markers cannot be regarded as direct evidence of immunomodulation. The antioxidant effects of microalgae may contribute to limiting subclinical inflammation; however, without functional immunological assays, claims of immune enhancement remain speculative.

**Role of microalgae in immune modulation and gut health improvement in poultry birds (Sonkusale. 2022).**

Microalgae strain	Immunomodulation function and role in gut health improvement
<b>Spirulina sp.</b>	Enhanced phyto-haemagglutinin-mediated propagation of lymphocytes and increased macrophage phagocytosis; supports development of lymphoid organs.
<b>Spirulina platensis</b>	Increased leukocyte count and macrophage phagocytic activity in H5N1-infected chickens; exhibited antimicrobial, immunomodulatory, anti-inflammatory and antioxidant activities; increased antibody titres against NDV in layers; increased interferon protein levels in birds dosed with sheep red blood cells (SRBCs).
<b>Chlorella sp.</b>	Enhanced phyto-haemagglutinin reaction stimulating T-cell metabolic activity and proliferation in broilers; supplementation via drinking water increased antibody titres against SRBCs (IgM and IgG) in laying hens; used as a replacement for antibiotic growth promoters with improvements in immune indices and intestinal microflora.
<b>Algal <math>\beta</math>-glucan</b>	Improved cell-mediated immune response through modulation of macrophage activity; elevated NDV-specific antibody titres following supplementation.
<b>Chlorella stigmatophora / Phaeodactylum tricornutum</b>	Extracts reported to exhibit anti-inflammatory, analgesic and free radical scavenging activities.
<b>Staurosira sp.</b>	Partial replacement of soybean meal with microalgae in feed showed no adverse effects on growth performance, plasma biomarkers or liver biomarkers.
<b>Haematococcus pluvialis</b>	Partial dietary replacement reduced caecal <i>Clostridium perfringens</i> counts.

With respect to species-specific differences, it is evident that most evidence for immunomodulatory effects of microalgae originates from experiments conducted in poultry. In pigs, immune parameters are monitored less frequently and are typically included only as ancillary measurements in studies primarily focused on growth performance and gut health. In ruminants, immune function is assessed only marginally in the available literature, most often indirectly through changes in metabolic or fermentation parameters. An exception may be studies investigating supplementation with DHA-rich microalgae, where the potential anti-inflammatory effects of n-3 polyunsaturated fatty acids are discussed; nevertheless, direct measurements of immune function are largely lacking even in these cases.

From an applied perspective, it is also important to distinguish whether immunomodulatory effects are attributed to microalgae as a complex matrix or to specific groups of bioactive compounds. The literature predominantly follows several interpretative lines:

- immunomodulation mediated by n-3 polyunsaturated fatty acids, particularly DHA, with potential anti-inflammatory effects;

- effects of pigments and antioxidants (e.g. phycocyanin, carotenoids) that may attenuate oxidative stress and secondarily influence inflammatory processes;
- actions of structural cell wall polysaccharides, which may interact with innate immune receptors and simultaneously modulate the gut microbiome.

Without clear identification of the active component(s), however, it is difficult to design targeted feed additives with reproducible effects. Many studies rely on whole microalgal biomass, making it challenging to disentangle whether observed effects are related to specific bioactive compounds or to general nutritional changes in the diet.

Overall, the available literature suggests a potential for microalgae to influence the immune system of livestock, but the strength of this evidence remains limited. The predominance of studies based on indirect immunological indicators, short experimental durations, and the absence of challenge models that would allow validation of the functional relevance of observed changes represent major limitations. To move microalgae from the domain of experimental nutrition towards genuinely functional immunomodulatory feed additives, future research must focus on comprehensive assessment of local intestinal immunity, functional immune responses, and their relationship with changes in the gut microbiome.

#### **4.4 Effects of Microalgae on the Intestinal and Rumen Microbiome**

In the contemporary concept of livestock nutrition, the gut microbiome is regarded as a key functional ecosystem mediating the relationship between diet, gut health, and host immune responses. Any dietary intervention that alters the composition or metabolic activity of the microbial community may have profound effects on the production of microbial metabolites, the integrity of the intestinal barrier, and the inflammatory status of the organism. In this context, microalgae are being investigated as potential modulators of the microbiome, both in monogastric animals and in ruminants, where it is essential to distinguish between the intestinal microbiome in the strict sense and the specific ecosystem of the rumen.

From a mechanistic perspective, microalgae are expected to influence the microbiome primarily through indirect pathways, via components that are not fully digestible by host enzymes but can be fermented by microorganisms. Structural polysaccharides of the microalgal cell wall, as well as other complex carbohydrates and proteins embedded within the cellular matrix, may function as selective substrates for specific microbial groups. In addition, microalgae may supply bioactive lipids or secondary metabolites that affect microbial growth and interactions within the microbial community.

##### **4.4.1 Intestinal Microbiome in Monogastric Animals**

In monogastric animals, particularly pigs and poultry, a relatively limited but methodologically relevant body of studies exists that attempts to directly assess the effects of microalgae on the composition of the intestinal microbiota. Studies in weaned piglets supplemented with *Chlorella vulgaris* represent some of the few investigations that link microbial community analysis with parameters of gut health and growth performance (Pestana et al., 2022). The authors demonstrate that inclusion of the microalga can lead to changes in microbiota composition without negatively affecting growth parameters, and interpret these changes as potentially beneficial for the intestinal environment.

An important aspect of this work is that the effect of microalgae on the microbiome is not evaluated in isolation but in the context of nutrient digestibility and availability. The authors point out that the cell wall of *C. vulgaris* may limit the release of certain components in the upper sections of the gastrointestinal tract, thereby increasing the likelihood of their fermentation in distal intestinal segments. This mechanism may explain the observed changes in microbial community structure; however, it simultaneously complicates interpretation as to whether the effect represents a targeted prebiotic action or rather a secondary consequence of reduced digestibility of specific dietary fractions.

In poultry, modulation of the microbiome by microalgae is frequently discussed in the context of replacing antibiotic growth promoters or conventional prebiotics. Studies framing *Chlorella vulgaris* as a prebiotic alternative are based on the assumption that microalgae may promote the growth of “beneficial” bacterial groups while limiting the proliferation of pathogens (Ahmed et al., 2025). Review articles focusing on poultry summarise results from multiple studies in which microalgae supplementation was associated with changes in intestinal microbiota composition, often with emphasis on the relative abundance of genera considered favourable for the host (Abd El-Hack et al., 2022; Van Nerom et al., 2024).

It must be critically emphasised, however, that most of these studies rely primarily on taxonomic descriptions of the microbial community, whereas the functional consequences of the observed changes are assessed only indirectly. A shift in the relative abundance of a given genus or family alone does not provide information on the metabolic activity of the microbiome or its impact on the host. Without concurrent measurements of short-chain fatty acid production, changes in intestinal content pH, or markers of intestinal barrier function, the functional significance of taxonomic shifts remains largely hypothetical.

#### 4.4.2 Rumen Microbiome in Ruminants: In Vivo Studies

In ruminants, the situation is methodologically distinct, as the rumen represents a highly complex fermentation system whose microbial composition and activity directly affect nutrient utilisation, energy production, and methane emissions. Several in vivo studies included in the analysed body of literature explicitly focus on changes in rumen microbiota in response to microalgae supplementation, particularly species of the genera *Schizochytrium* and *Spirulina*.

A study conducted in goats evaluating different levels of *Schizochytrium* spp. supplementation provides valuable data on changes in particle-associated rumen microbiota, including both bacterial and archaeal populations (Mavrommatis et al., 2021). The authors demonstrate that the inclusion level of the microalgae significantly influences the structure of the microbial community, including groups associated with fibre degradation and methanogenesis. These changes are interpreted in the context of a potential reduction in methane production and modification of fermentation patterns, with direct implications for feed utilisation efficiency as well as the environmental footprint of livestock production.

Similarly, a study investigating *Spirulina* supplementation in ewes documents shifts in rumen microbiota composition and suggests that even relatively short-term dietary interventions may lead to detectable changes in the rumen microbial ecosystem (Mancini et al., 2023). Together, these studies provide important evidence that microalgae can act as modulators of the rumen microbiome; however, their relevance to “gut health” in the narrower sense remains indirect.

From an interpretative standpoint, it is crucial to emphasise that changes in rumen microbiota cannot be automatically extrapolated to conclusions regarding the intestinal microbiome of post-ruminal sections of the gastrointestinal tract. Nevertheless, these studies offer an important methodological framework for investigating interactions between microalgae and microbial communities, particularly with respect to dose-dependent effects and targeted monitoring of functionally relevant microbial groups.

#### **4.4.3 In Vitro Fermentation Studies and Their Limitations**

A substantial proportion of the available literature consists of in vitro rumen fermentation studies evaluating the effects of different microalgal species on gas production, volatile fatty acid profiles, and methane output (Sucu, 2020; additional in vitro studies included in the analysed dataset). These studies are valuable as screening tools, enabling rapid comparison of different microalgal species and identification of potentially promising candidates for subsequent in vivo evaluation.

However, it is essential to clearly delineate their limitations. In vitro systems lack host-mediated regulatory mechanisms, exhibit limited temporal dynamics, and often employ microalgal inclusion levels that are unrealistic from a practical feeding perspective. Consequently, results obtained from in vitro studies cannot be regarded as direct evidence of microalgal effects on the microbiome in living animals, but rather as sources of hypotheses regarding potential modes of action.

#### **4.4.4 Critical Synthesis**

Overall, it can be concluded that microalgae demonstrably have the capacity to influence microbial communities in the gastrointestinal tract of livestock. The most convincing evidence originates from in vivo studies focusing on the rumen microbiome in ruminants and from a limited number of studies in monogastric animals that combine microbiota analysis with assessment of gut health parameters. A major limitation of the current literature, however, is the frequent separation of taxonomic descriptions of the microbiome from functional and host-related parameters. Without integration of data on microbial activity, metabolite production, and host responses, it remains difficult to formulate unequivocal conclusions regarding the benefits of microalgae as targeted modulators of the intestinal microbiome.

### **4.5 Integrated Discussion: Linking Gut Health, Immunity, and the Microbiome in the Context of Microalgae Utilisation**

The available literature on the use of microalgae in livestock feeds suggests that the potential benefits of these resources cannot be evaluated in isolation within individual biological systems. Gut health, immune responses, and the composition of the intestinal microbiome form a functionally interconnected network, in which changes in one domain may substantially influence the others. Although this interconnection is frequently acknowledged in the literature, it is only rarely tested systematically within a single experimental design.

From the perspective of gut health, microalgae can be viewed as dietary interventions capable of affecting both the structural properties of the intestinal mucosa and the chemical and microbial environment of the intestinal lumen. In monogastric animals, there are indications that microalgae

supplementation may lead to changes in intestinal morphology and improvements in certain indicators of the gut environment; however, these changes are often evaluated independently of immune and microbiological parameters. As a result, it remains unclear whether the observed effects represent a primary action of microalgae on the intestinal epithelium or a secondary adaptation to altered microbial composition or fermentative activity.

The immune system, particularly intestinal mucosal immunity, represents a key linking element between diet and the microbiome. Microalgae are frequently presented in the literature as immunomodulatory feed components; however, the prevailing evidence is largely based on indirect indicators, such as antioxidant markers or changes in circulating immunological parameters. Direct assessment of local immune responses in the gut—such as measurements of secretory IgA, cytokine profiles, or histological evaluation of inflammatory changes—is rare in the available studies. This significantly limits the ability to draw conclusions regarding the true functional relevance of the reported immunological changes.

In this context, the intestinal microbiome appears to be a central mediator of microalgal effects, yet its role is often reduced in the literature to a descriptive, taxonomic level. Numerous studies document changes in microbial community composition following microalgae supplementation, but only rarely link these changes to functional outputs relevant to host health. Shifts in the relative abundance of specific bacterial groups are frequently interpreted as “positive” or “negative” without clear justification based on microbial metabolic activity or interactions with the immune system.

Generalisation of results obtained from in vitro studies or from the rumen microbiome of ruminants to the concept of gut health in monogastric animals is particularly problematic. Although the rumen represents a unique model for studying dietary modulation of microbial communities, its functional role and host–microbe interactions differ fundamentally from those in the small and large intestine of monogastric species. Nevertheless, conclusions regarding a “positive effect on the microbiome” are sometimes implicitly transferred between these systems without clearly defining their biological limitations.

Another important factor complicating integration of existing knowledge is the heterogeneity of microalgal species and processing methods used across studies. The literature encompasses a wide range of microalgal species that differ markedly in chemical composition, cell wall structure, and content of bioactive compounds. In addition, processing methods vary substantially, ranging from dried whole biomass to partially disrupted or extracted forms. These differences have a major impact on the bioavailability of individual components and, consequently, on their potential effects on gut health, immunity, and the microbiome. Despite this, results from individual studies are often discussed collectively, without sufficient consideration of these technological aspects.

From an integrative perspective, several recurring patterns can be identified in the literature:

- effects of microalgae on gut health and immunity are often mediated by changes in the intestinal microbiome, yet this relationship is rarely tested directly;
- positive effects are more frequently observed under conditions of stress or suboptimal health status, whereas effects in healthy animals receiving balanced diets tend to be less pronounced;
- higher inclusion levels of microalgae are more likely to induce measurable biological changes, but this increasingly blurs the distinction between feed additives and feed ingredients.

These patterns suggest that the potential of microalgae as functional feed additives is strongly context-dependent. The effects of microalgae cannot be evaluated in isolation but must always be

interpreted in relation to overall diet composition, the physiological status of the animal, and specific production conditions. This has important implications for both the design of future experiments and the practical application of microalgae in animal nutrition.

From a critical standpoint, it is also necessary to highlight the risk of overestimating the effects of microalgae based on limited or indirect evidence. In some cases, microalgae are attributed complex health benefits without clear demonstration of causal links between dietary intervention, changes in the microbiome, immune responses, and clinical outcomes. To advance this field towards a more robust scientific foundation, it is essential to move beyond isolated evaluation of individual parameters and to adopt integrated experimental approaches that allow comprehensive assessment of the effects of microalgae on animal health.

## **4.6 Recommendations for Future Research and Applied Use of Microalgae in Animal Feeds**

A critical evaluation of the available literature indicates that microalgae represent a promising yet not fully exploited tool for targeted modulation of gut health, immune function, and the intestinal microbiome in livestock. At the same time, it reveals a number of methodological and interpretative weaknesses that limit the transfer of experimental findings into practical application. Further progress in this field therefore requires systematic and targeted research that reflects both the biological complexity of the studied systems and the realities of livestock production.

### **Experimental Design and Model Selection**

One of the key shortcomings of current studies is the lack of comprehensive experimental designs that simultaneously assess gut health parameters, immune responses, and the microbiome. Future research should prioritise integrated approaches that enable identification of causal relationships between dietary interventions and host biological responses. This includes in particular:

- the use of in vivo models with clearly defined physiological or production-related stress, such as the weaning period in piglets, heat stress in poultry, or high production load in ruminants;
- incorporation of challenge models that allow verification of the functional relevance of microalgae supplementation, for example through infectious pressure, vaccination, or dietary stress;
- consistent differentiation between studies investigating microalgae as feed ingredients and those evaluating their potential as feed additives.

### **Selection of Appropriate Parameters and Endpoints**

Assessment of the effects of microalgae on gut health and immunity requires moving beyond a reduced approach based solely on growth and production parameters. Future studies should include a standardised set of biologically relevant endpoints enabling comprehensive evaluation of effects. In the area of gut health, this should include in particular:

- morphological parameters of the intestinal mucosa (villus height, crypt depth, villus-to-crypt ratio) combined with histological assessment of inflammatory changes;
- functional indicators of intestinal barrier integrity, such as permeability assays or evaluation of tight junction protein expression;
- chemical and fermentative parameters of intestinal contents, including concentrations of short-chain fatty acids and pH.

In the field of immunity, it is essential to focus on functional indicators that reflect true immune competence of the animal. This includes:

- assessment of local intestinal immunity, particularly secretory IgA production and cytokine expression in the intestinal mucosa;
- monitoring immune responses to vaccination or infectious challenge;
- integration of systemic and local immunological parameters with clinical indicators of health status.

### **Integration of Microbiome Data**

Microbiome analysis should form an integral part of future studies, but it must be designed to provide not only descriptive but also functionally relevant information. This requires:

- combining taxonomic profiling of microbial communities with measurement of microbial metabolites, particularly short-chain fatty acids;
- integrating microbiome data with host response parameters, especially changes in intestinal barrier function and immune responses;
- in ruminants, clearly defining whether the objective is modulation of rumen fermentation or the broader concept of digestive tract health.

### **Characterisation and Processing of Microalgae**

A key factor determining the biological efficacy of microalgae is their chemical composition and processing method. Future studies should therefore place strong emphasis on detailed characterisation of the microalgae used, including:

- basic chemical composition (proteins, lipids, polysaccharides, minerals);
- content of key bioactive components, such as polyunsaturated fatty acids, pigments, or specific polysaccharides;
- biomass stability, particularly in lipid-rich microalgae where oxidation may markedly influence biological effects.

At the same time, systematic evaluation of the impact of technological processing—such as mechanical cell wall disruption or the use of enzymatic preparations—on bioavailability of active components is highly desirable. These aspects are critical for the development of effective feed additives with reproducible effects.

### **Applied Perspective and Practical Relevance**

From an applied perspective, it is essential that future research reflects real-world livestock production conditions. This includes not only realistic inclusion levels of microalgae but also assessment of their economic feasibility and compatibility with existing feeding systems. For practical use of microalgae as feed additives, it is particularly important to:

- define the minimum effective dose for specific purposes and animal species;
- assess long-term effects of supplementation, including potential adaptive changes in the microbiome;
- take into account regulatory frameworks and feed safety requirements.

Overall, it can be concluded that the potential of microalgae as functional feed additives is considerable, but its full exploitation requires a shift from fragmented experimental approaches towards systematic, integrated research. Only through such an approach will it be possible to formulate scientifically robust recommendations and effectively translate experimental knowledge into practical application.

## **4.7 Conclusion**

The aim of this review was to critically evaluate the available scientific evidence on the use of microalgae in feeds for livestock, with a focus on three closely interconnected areas: gut health, immune responses, and modulation of the intestinal microbiome. Analysis of experimental and review studies indicates that microalgae represent a biologically active and technologically promising group of feed resources; however, their effects cannot be assessed uniformly or without consideration of animal species, processing form, and mode of inclusion in the diet.

With respect to gut health, more convincing evidence is available primarily for monogastric animals, particularly poultry and pigs, where several studies document changes in intestinal mucosal morphology, parameters of the intestinal environment, and associated shifts in microbial communities. Nevertheless, these findings are often based on a limited set of measured indicators and are not consistently supported by functional assessments of intestinal barrier integrity or clinically relevant manifestations of gut health. In ruminants, the concept of “gut health” in the available literature is generally replaced by evaluation of rumen fermentation stability, which has clear biological and practical relevance but cannot be directly equated with intestinal health in the narrower sense.

Immunomodulatory effects of microalgae are frequently cited in the literature; however, their experimental support remains relatively weak. Most studies rely on indirect immunological indicators, particularly antioxidant markers or changes in circulating immune parameters, whereas direct assessment of local intestinal immunity and functional immune responses is rare. This limits the ability to unequivocally conclude that microalgae act as true immunomodulatory feed additives rather than merely as nutritionally valuable dietary components with secondary beneficial effects.

The strongest and methodologically most robust body of evidence relates to modulation of microbial communities in the gastrointestinal tract, especially in ruminants, where *in vivo* studies demonstrate that microalgae supplementation can lead to measurable changes in the rumen microbiome and

fermentation processes. In monogastric animals, evidence for alterations of the intestinal microbiome is more limited and often based on taxonomic descriptions without sufficient functional interpretation. Nevertheless, it is evident that the microbiome represents a key mediator of microalgal effects on both gut health and immune function and should therefore constitute an integral component of future research.

From an integrative perspective, it can be concluded that the potential of microalgae as functional feed additives is real but strongly context-dependent. Observed effects are often the result of complex interactions among diet composition, the microbial ecosystem, and host responses, rather than direct action of a single bioactive compound. This has important implications for interpretation of experimental data and for formulation of application-oriented recommendations.

At the same time, it must be emphasised that a substantial proportion of the literature is characterised by a fragmented approach, in which individual biological systems are evaluated in isolation. Without integrated experimental designs linking gut health, immunity, and the microbiome within a single model, conclusions regarding the functional benefits of microalgae often remain incomplete or speculative. Future research should therefore focus on comprehensive assessment of microalgal effects under realistic production conditions, with emphasis on functional endpoints and long-term reproducibility of results.

In conclusion, microalgae represent a promising yet not fully exploited component of modern animal nutrition. Their successful integration into feeding strategies aimed at supporting gut health, immune function, and the microbiome will depend on the ability of research to bridge the gap between experimental findings and practical application requirements. Only on the basis of robust, integrated, and critically interpreted data will it be possible to formulate scientifically sound claims and effectively harness the potential of microalgae as functional feed additives.

## 4.8 Summary of Key Findings

### Which microalgae species were used?

Across the reviewed studies, research focused predominantly on a limited number of freshwater microalgae species. The most frequently investigated taxa were:

- *Chlorella vulgaris*
- *Spirulina platensis* (syn. *Arthrospira platensis*)

In addition, several studies—particularly in poultry—investigated mesobiliverdin IX $\alpha$  (MBV)-enriched microalgal biomass, most commonly derived from Spirulina.

Other microalgal taxa (e.g. *Nannochloropsis*, *Schizochytrium*, *Tetraselmis*, *Isochrysis*) were included in some studies, but these were primarily focused on rumen fermentation, lipid metabolism, or methane mitigation in ruminants rather than gut health outcomes in poultry or pigs.

Overall, no broad or systematic evaluation of diverse microalgal taxa for gut health effects in monogastric animals was identified.

### **Was biomass or an extract used?**

The reviewed studies predominantly employed whole microalgal biomass, which was incorporated directly into experimental diets.

A smaller subset of studies used processed or enriched biomass preparations, most notably MBV-enriched microalgae. These products represent modified biomass rather than purified extracts.

Importantly:

- No studies used isolated, purified algal compounds as standalone feed additives.
- MBV-enriched preparations still relied on microalgal biomass as the delivery matrix, not on purified MBV.

Thus, the available evidence is based almost exclusively on biomass-based interventions.

### **What was the concentration/amount of the algal component in the diet?**

Microalgae inclusion levels varied depending on the animal species, microalgal product, and study design.

#### ***Broiler chickens***

- Typical inclusion levels ranged from 0.25 to 3% of the diet
- Spirulina was most commonly applied at 1–2 g/kg feed
- MBV-enriched microalgae were used at lower levels, typically 0.05–0.1% of the diet
- Higher inclusion levels (up to ~3%) were often associated with measurable improvements in gut morphology and performance

#### ***Weaned piglets***

- Most studies applied inclusion levels around 1% of the diet
- MBV-enriched microalgae were used at 0.1–0.5%
- High inclusion levels of whole biomass (e.g. ≥5% Chlorella vulgaris) were associated with reduced nutrient digestibility and, in some cases, adverse physiological responses, limiting their practical relevance

Overall, the data indicate species-specific tolerance and response, with broilers generally tolerating and responding positively to higher inclusion levels than pigs.

### **What technology was used for incorporating microalgae into the product?**

All reviewed studies incorporated microalgae through direct dietary supplementation, typically by:

- Mixing dried microalgal biomass into compound feed
- Applying standard feed manufacturing processes

No advanced delivery or protection technologies were reported.

- Some studies included co-interventions, such as:

- Antibiotics (e.g. tylosin, colistin, amoxicillin) as positive controls
- Probiotics
- Carbohydrase enzymes (e.g. xylanase)

However, no novel processing technologies (e.g. microencapsulation, fermentation-based incorporation, or targeted delivery systems) were described.

### **What were the results concerning gut health?**

Microalgae supplementation was frequently associated with improvements in gut health-related parameters, particularly in poultry and, to a lesser extent, in pigs. Reported effects included:

- Increased villus height and improved villus height-to-crypt depth ratios
- Improved intestinal morphology in the duodenum, jejunum, and ileum
- Reduced incidence or severity of post-weaning diarrhea in piglets (mainly with *Chlorella vulgaris*)
- Improved markers related to gut barrier integrity (in a limited number of studies)
- Reduced expression of pro-inflammatory markers and lower oxidative stress indicators (e.g. MDA, TBARS)

While these findings suggest a positive effect on gut health, it should be noted that direct functional measurements of barrier integrity and inflammation were not consistently applied across studies.

### **What were the results concerning the intestinal microbiome?**

Microalgae supplementation resulted in selective modulation of the intestinal microbiota, rather than broad restructuring.

Commonly reported effects included:

- Increased abundance of potentially beneficial bacterial taxa, such as:
  - *Lactobacillus* spp.
  - *Bifidobacterium* spp. (especially when combined with probiotics)
  - *Oscillospira* and *Colidextribacter* in piglets
  - Reduced *Escherichia coli* counts in some broiler studies
  - Changes in the Firmicutes/Bacteroidetes ratio in poultry

Across studies:

- Alpha diversity indices (e.g. Shannon, Chao1) were generally unchanged
- Microbiome effects appeared taxon-specific rather than global

These results are consistent with a prebiotic-like mode of action.

### **What were the results concerning antiparasitic effects?**

No direct antiparasitic effects were demonstrated.

Specifically:

- No studies employed experimental parasite challenge models
- No parasite load measurements were conducted
- No helminths, protozoa (e.g. *Eimeria*), or ectoparasites were directly assessed

Observed reductions in diarrhea were attributed to improved digestive stability and microbiota modulation, not to antiparasitic activity.

Therefore, current evidence does not support claims of antiparasitic efficacy of microalgae in poultry or pigs.

### **Were health benefits observed?**

Yes, consistent health-related benefits were reported, particularly in relation to:

- Intestinal morphology and integrity
- Inflammatory and oxidative stress status
- Modulation of intestinal microbiota
- Reduction of digestive disturbances (notably post-weaning diarrhea)

Effects on growth performance were species- and dose-dependent:

- Minimal or inconsistent in weaned piglets
- Variable to clearly positive in broiler chickens, especially at moderate-to-higher inclusion levels

### **Other information relevant to our project**

Several findings are particularly relevant for applied research and feed additive development:

- Dose optimisation is critical, as beneficial effects may diminish or reverse at excessive inclusion levels
- MBV-enriched microalgae show enhanced anti-inflammatory and gut-protective potential
- Microalgae act via multifactorial mechanisms (antioxidant, anti-inflammatory, prebiotic, barrier-supporting)
- Current evidence supports positioning microalgae as gut health and microbiome modulators, not as antiparasitic agents

Key research gaps include:

- Lack of parasite challenge studies
- Limited data on long-term supplementation
- Insufficient standardisation of gut health and microbiome endpoints

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## 5 PROJECTS



<https://cordis.europa.eu/project/id/862980>

**Project title:** ProFuture – Microalgae Protein-Rich Ingredients for the Food and Feed of the Future

**Programme:** Horizon 2020

**Objective:**

To improve the production and utilisation of protein-rich ingredients derived from microalgae for food and feed applications and to support their market uptake.

**Brief description:**

The project focused on increasing the efficiency of microalgae production, developing protein-rich ingredients, and demonstrating their use in pilot-scale food and feed applications.

**Key outputs:**

- production and processing methodologies for microalgal biomass;
- technological concepts for integration of microalgae-derived ingredients into feed and food products.

**Summary:**

Microalgae represent a promising source of protein-rich ingredients for food and feed applications, characterised by relatively low energy and water requirements. However, their broader utilisation has so far been constrained by high production and processing costs of microalgal biomass. The ProFuture project addressed these challenges by evaluating and developing advanced production systems for the efficient generation of single-cell proteins and protein isolates from microalgae. These products were characterised in terms of their nutritional properties and economic sustainability. Selected protein fractions were tested in food and feed applications at an industrial scale. The project also included an analysis of the microalgae value chain within the EU and proposed measures to enhance the competitiveness of the sector, including support for market uptake through targeted dissemination and implementation activities.

<https://www.pro-future.eu/>



<https://cordis.europa.eu/project/id/727874>

**Project title:** SABANA – Sustainable Algae Biorefinery for Agriculture and Aquaculture

**Programme:** Horizon 2020

**Objective:**

To develop a sustainable microalgae-based biorefinery platform for the production of feed and aquaculture additives, integrated with nutrient recycling from wastewater streams.

**Brief description:**

The project combined research and large-scale demonstration of integrated microalgae systems for biomass production and its subsequent valorisation into products for agriculture, animal feed, and aquaculture.

**Key outputs:**

- development of an integrated microalgae-based biorefinery concept;
- production of feed additives, aquafeed, biostimulants, biopesticides, and biofertilisers;
- demonstration of nutrient recovery from wastewater streams and manure;
- validation of sustainability and circular economy approaches for agri-food systems.

**Summary:**

Wastewater can represent a valuable resource rather than an environmental burden. The EU-funded SABANA project aimed to revolutionise the bioproduct industry by developing a large-scale, integrated microalgae-based biorefinery using marine water and nutrients recovered from wastewater streams. The project focused on the production of a broad portfolio of bioproducts, including feed additives, aquafeed, biostimulants, biopesticides, and biofertilisers. By recovering nutrients from sewage, centrate, and pig manure, SABANA sought to minimise waste generation and energy consumption while closing nutrient loops. The project addressed the urgent need for sustainable solutions in agriculture and aquaculture by delivering microalgae-derived products designed to enhance crop productivity and fish production within a circular bioeconomy framework.

## MULTI-STR3AM

<https://cordis.europa.eu/project/id/887227>

**Project title:** MULTI-STR3AM

**Programme:** Horizon 2020 – BBI JU (Bio-Based Industries Joint Undertaking)

**Objective:**

To integrate multiple microalgae biorefinery technologies enabling the production of several value streams, including ingredients for food, feed, and fragrance applications.

**Brief description:**

The project focused on the development and demonstration of an integrated microalgae biorefinery platform combining different cultivation systems, biomass processing routes, and extraction technologies to enable flexible valorisation of microalgal biomass.

**Key outputs:**

- design and validation of a multi-stream microalgae biorefinery concept;
- integration of cultivation, harvesting, and downstream processing technologies;
- development of multiple product streams, including fractions relevant for feed and food applications;
- techno-economic and sustainability assessment of integrated biorefinery approaches.

**Summary:**

Current agricultural and manufacturing practices are causing irreparable environmental damage. Although microalgae are a promising solution to this problem, they are underexploited as a crop. This is because microalgae products struggle to achieve the same economies of scale as conventional products. The EU-funded MULTI-STR3AM project aims to provide valuable microalgal products for large end users in the food, feed and fragrance sectors by reducing costs, increasing scale and boosting sustainability. Its products will include lipids for edible spreads, protein, carbohydrates and lipids for feed ingredients for poultry, pigs and ruminants, and protein and small organic compounds as building blocks for the fragrance industry. The project will pave the way for sustainable industrial-scale microalgae cultivation in Europe.



<https://cordis.europa.eu/project/id/745754>

**Project title:** MAGNIFICENT – Microalgae As a Green Source for Nutritional Ingredients

**Programme:** EU-funded project (launched in 2025)

**Objective:**

To optimise microalgae as sustainable sources of nutritional ingredients, with a focus on high-value products and comprehensive socio-economic assessment of their production and use.

**Brief description:**

The project focuses on market analysis, optimisation of microalgae production systems, and development of novel applications of microalgae-derived ingredients, including their use in the feed sector.

**Key outputs (expected):**

- optimisation strategies for microalgae-based nutritional ingredients;
- identification and validation of high-value product streams;
- techno-economic and socio-economic assessments supporting market uptake;
- evaluation of food and feed applications of microalgae-derived ingredients.

**Summary:**

In addition to being rich in vitamins, soluble fibres, proteins, lipids and minerals, algae, including microalgae and seaweed, also serve as abundant sources of minerals and vitamins. Their unique properties make them ideal solutions for sustainable food ingredients, animal feeds and cost-effective cosmetics. However, algae are currently utilised in limited quantities due to their high production costs, which hinder their competitiveness. The EU-funded MAGNIFICENT project aims to address this by offering innovative products and applications to fully leverage the opportunities presented by algae. To achieve this goal, MAGNIFICENT will enhance and optimise production processes, develop and validate new product formulations, and conduct thorough chain evaluation, market assessment, socio-economic impact assessment and life-cycle assessment.

<https://magnificent-algae.eu/>

## ALLIANCE

<https://cordis.europa.eu/project/id/101214199>

**Project title:** ALLIANCE – European Microalgae Alliance

**Programme:** Horizon Europe

### **Objective:**

To expand the use and market uptake of microalgae-derived products within the EU, including applications in aquafeed and agriculture, with a strong focus on cost-effectiveness and sustainability.

### **Brief description:**

The project focuses on improving the economic viability and sustainability of microalgae production, including the development of biorefinery-based solutions enabling multiple product streams, among them feed-relevant raw materials.

### **Key outputs (expected):**

- strategies to improve cost-effectiveness of microalgae production systems;
- development and validation of sustainable microalgae biorefinery concepts;
- support for market deployment of microalgae-derived products in aquafeed and agricultural sectors;
- strengthening of cooperation across the European microalgae value chain.

### **Summary:**

Microalgae products can introduce sustainability in the food sector. With this in mind, the EU-funded ALLIANCE project will promote the adoption of microalgae-based products in the EU market, improving cost-effectiveness, circularity and overall sustainability. It will establish algae-based mid-value ingredients using multi-product biorefineries for the food, cellular agriculture, aquafeed and agricultural sectors, making these products more affordable and sustainable. Project goals include nutrient and water recirculation, automated production controls, and a focus on renewable energy and solvent-free processes. The project will also integrate upstream and downstream processes, expanding from four to 15 product lines with minimal waste, and employ scalable, solvent-free extraction and purification technologies using wet biomass.



<https://cordis.europa.eu/project/id/101060607>

**Project title:** CIRCALGAE

**Programme:** Horizon Europe

### **Objective:**

To develop sustainable microalgae-based biorefineries and produce high-value ingredients for feed, food, and cosmetic applications within a circular bioeconomy framework.

**Brief description:**

The project focuses on valorisation of microalgal biomass, including industrial algae side streams, and on the production of ingredients applicable in animal nutrition, food, and cosmetics.

**Key outputs (expected):**

- development of an integrated, water-based microalgae biorefinery concept;
- valorisation of algae biomass and industrial algae side streams;
- production of novel macro- and microalgae-derived ingredients for feed, food, and cosmetic sectors;
- contribution to circular and climate-positive bioeconomy solutions.

**Summary:**

Algae biomass is a treasure in the water, while capturing CO<sub>2</sub> vast amounts of biomass are generated. However, this treasure is not being used to the fullest. The EU-funded CIRCALGAE project aims to develop a ground-breaking exploitation system where the algae industrial waste streams will be further valorised, in a more sustainable and circular approach. Through a set of simple, water-based technologies, the project focuses on introducing an integrated biorefinery concept and delivering novel macro- and microalgae ingredients to the food, feed and cosmetic sector. Bringing together a team of key algae cross-sectoral actors, CIRCALGAE aspires to revolutionise the blue bioeconomy and scale up the sustainable use of living aquatic resources.

<https://www.circalga.eu/>

## 6 PATENT OVERVIEW

### 6.1 Context and Methodology of the Patent Landscape Analysis

The patent landscape analysis focused on the use of microalgae in livestock nutrition revealed that microalgae appear relatively frequently in patent literature; however, in most cases their role is limited to one of several components within complex feed formulations. These patents typically describe feed compositions, proportions of individual ingredients, and feed manufacturing processes, with microalgae used as a general nutritional or functional component without further specification of biologically active compounds or mechanisms of action.

During the patent search, it became evident that the formulation of search queries has a decisive impact on the nature of the results obtained. Broadly defined queries generate very large patent datasets that are largely irrelevant to the objectives of this study, as they focus primarily on feed formulations and application-related details. In contrast, targeted queries focusing on gut health, the intestinal microbiome, and immune function in livestock—combined with the exclusion of vaccines, pharmaceutical applications, and formulation-based patents—result in significantly smaller but substantially more relevant patent sets.

For this reason, the patent analysis was designed as an iterative process. For the purposes of this report, only patents addressing microalgae as sources of functional compounds or biologically active fractions with claimed effects on gut health, the intestinal microbiome, or the immune system of livestock were analysed. In future work, it will be appropriate to regularly update the patent

landscape in response to target compounds identified through research, specific health challenges addressed by the project (e.g. post-weaning diarrhoea, dysbiosis), and the target animal species.

### **Exclusion of Patents Focused Exclusively on Feed Formulations**

Patents whose primary subject of protection consists of feed mixtures, their composition, and the ratios of individual ingredients were excluded from further analysis. Although these patents often include microalgae or microalgae-derived products, they do so without identification of specific bioactive components and without description of biological mechanisms of action relevant to gut health, the microbiome, or immune function.

These so-called formulation patents were therefore considered of limited relevance to the objectives of this study, which aims to identify transferable biological principles and functional compounds applicable in feed additives rather than to optimise specific feed formulations. As such, formulation patents serve primarily as evidence of general interest in microalgae within the livestock nutrition sector, rather than as sources of technologically or biologically specific information.

## **6.2 Detailní rozbor vybraných patentů (priorita: zdraví střeva a prebiotický efekt)**

### **CN108991259A – Pig feed additive for reducing diarrhea of piglets**

This patent focuses on the use of a feed additive for piglets with the objective of reducing the incidence of diarrhoeal diseases. The abstract explicitly states a positive effect on gut health and stabilisation of the intestinal environment, with a microalgae-derived component identified as one of the key functional elements of the additive. A particularly relevant aspect is the declared ability to influence intestinal microbial balance, suggesting a potential prebiotic mechanism of action.

In contrast to purely formulation-based patents, microalgae are not presented here merely as a nutritional ingredient but as a functional component contributing to the reduction of intestinal disorders during the sensitive post-weaning period. The patent is therefore highly relevant from the perspective of applications targeting gut health in piglets and prevention of dysbiosis, which aligns well with the priorities of this study. Although the abstract does not fully specify the nature of the active component, the patent represents one of the few examples in which a direct link between microalgae and gut health is explicitly articulated.

### **CN115024423A – Two-color natural nutritional supplement and preparation method thereof**

This patent describes a natural nutritional supplement combining the microalga *Nannochloropsis* with selected probiotic microorganisms. A key feature of this patent is that microalgae are not presented in isolation but within a framework of gut microbiota modulation, creating a concept closely aligned with synbiotic approaches. The abstract explicitly mentions positive effects on intestinal microbial balance and overall animal health.

From the perspective of this study, the patent is particularly relevant as an example of a technological approach in which microalgae are used as supportive or prebiotic components influencing the microbiome. Although the patent still relates to a complex supplement, the explicit emphasis on

microbiota modulation and the combination with probiotics distinguishes it from conventional formulation patents. The document clearly illustrates a broader trend towards positioning microalgae within the context of the intestinal ecosystem rather than merely as nutrient sources.

#### **US2022240542A1 – Maturation of immune and metabolic processes via algal biomass**

This patent addresses the administration of algal biomass or related materials to livestock with the aim of influencing maturation of immune and metabolic processes. The patent is based on the concept of microalgae as biologically active materials capable of modulating systemic processes, including immune functions. The application scope includes livestock species, such as poultry.

Although this patent is not primarily focused on gut health or microbiome modulation, it represents a relevant example of broader functional use of microalgae with biological effects beyond basic nutrition. Its inclusion in the report provides important contextual information, demonstrating that microalgae are perceived in patent literature not only as nutritional components but also as modulators of immune maturation and metabolic programming in animals.

#### **US10912805B2 – Extract of algae for use as an immunomodulator agent**

This patent protects the use of an algae-derived extract as an immunomodulatory agent. In contrast to patents based on whole biomass, this document focuses on an extract, indicating a higher level of technological specificity and potentially improved transferability to practical applications. The patent claims immunomodulatory effects without being restricted to a specific feed formulation.

Although the patent is not primarily targeted at gut health or microbiome modulation, it is relevant to this study as an example of an approach centred on an identifiable microalgae-derived fraction with defined biological activity. The document supports the argument that microalgae can serve as sources of functional compounds suitable for feed additives and provides a clear contrast to less specific formulation-based patents.

#### **CN118291571A – *Schizochytrium limacinum* peptide chelated selenium for piglets**

This patent focuses on the use of the microalga *Schizochytrium limacinum* as a source of a functional ingredient in the form of peptide-chelated selenium intended for piglets. The patent claims positive effects on health status and immune responses, with the microalga serving as the biological basis for generating a specific functional form of a trace element.

From the perspective of this study, the patent is particularly interesting because it works with a defined microalgal species and a clearly specified functional compound rather than with generic biomass. Although the primary emphasis is placed on immune effects, the document illustrates a trend towards technologically specific and biologically justified applications of microalgae in livestock nutrition.

### **6.3 Conclusions of the Patent Analysis and Relevance for the Project**

The patent analysis indicates that although microalgae are used relatively frequently in the field of livestock nutrition, their patent protection is in most cases limited to feed formulations and application-oriented solutions without deeper biological specification. Patents that explicitly focus on microalgae as sources of specific bioactive compounds with defined effects on gut health, the intestinal microbiome, or the immune system of livestock represent only a minority of the identified patent set.

In particular, patent activity related to gut health and the prebiotic effects of microalgae remains limited and is often based on declared functional effects without detailed elaboration of mechanisms of action or chemical characterisation of the active components. This suggests that, in current patent practice, microalgae are frequently applied empirically as complex biomass rather than as sources of specifically characterised prebiotic or immunomodulatory substances.

From the perspective of the project, this situation represents a significant opportunity. The identified gap between the relatively widespread practical use of microalgae in animal feeds and the limited number of patents addressing mechanistic understanding of their effects creates space for research focused on systematic identification of active microalgal components, their interactions with the intestinal microbiome, and their influence on intestinal barrier integrity and immune responses. Particularly promising areas include research on microalgal polysaccharides, cell wall structures, and other structural components that may act as prebiotics or modulators of the intestinal environment.

The patent review also demonstrates that existing patent solutions in this field are fragmented and terminologically inconsistent, which complicates systematic searching and comparison of existing technologies. This supports the need for continuous updating of the patent landscape throughout the project in response to refinement of target compounds, biological effects, and target animal categories. Such an iterative approach will enable not only monitoring of emerging patent activity but also more effective protection of the project's own results in areas where strong patent competition is currently lacking.

Overall, the results of the patent analysis confirm the relevance of the project's focus on microalgae as sources of functional compounds for supporting gut health and immune function in livestock, while also highlighting a largely underexploited innovation potential in this area, particularly in the context of prebiotic applications and mechanistically substantiated solutions.

## 7 PRODUCTS



**Company:** Allmicroalgae (product line: ALLVITAE)

**Headquarters / Country:** Rua 25 de Abril, Pataias-Gare, 2445-413 Pataias, Portugal

**Website:** <https://www.allmicroalgae.com/en/allvitae/>

### Products / Solutions:

ALLVITAE feed products – a range of 100% microalgae-based ingredients intended for animal feed, including applications for pigs and poultry. The products are positioned as functional feed ingredients supplying nutrients and bioactive compounds such as antioxidants, polysaccharides, and glycopeptides, with claimed relevance for intestinal health and immune support.

The portfolio includes microalgal species such as:

- *Chlorella vulgaris*
- *Nannochloropsis* spp.
- *Tetraselmis* spp.
- *Scenedesmus* spp.
- *Spirulina* (*Arthrospira* spp.)

Products are available in powder or paste forms, suitable for incorporation into premixes or compound feed formulations.

### Note:

The products are not always marketed under distinct commercial product names but are frequently supplied as **specific microalgal biomasses or fractions** intended for integration into premixes or customised feed formulations, depending on customer requirements.





**Company:** Phycom – *The Algae Creators*

**Headquarters / Country:** Koningsschot 11, 3905 PP Veenendaal, The Netherlands

**Website:** <https://phycom.eu/algae-by-phycom-for-animals/>

**Products / Solutions:**

Microalgae powders / biomass – microalgae-derived raw materials intended for use in animal feed formulations, including diets for piglets and chicks. The ingredients are positioned for applications targeting gut health improvement and immune system activation.

Phycom supplies microalgal biomass and powders as feed ingredients rather than finished compound feeds. The products are designed for integration into custom feed formulations and premixes developed by industrial feed manufacturers.

**Note:**

Phycom does not market branded, ready-to-use feed products. Instead, the company operates as an ingredient supplier, providing microalgae-based raw materials to support the development of functional feeds by third-party producers.



**Cargill – Feed Additives & Microbiome Solutions**

**Headquarters / Country:** Global company (headquarters in the USA), with significant European operations (Cargill Animal Nutrition)

**Website:** <https://www.cargill.com/animal-nutrition/species/feed-additives>

**Products / Solutions:**

A portfolio of feed additives targeting gut health and the microbiome, covering the pig and poultry segments. The portfolio includes probiotics, postbiotics, and organic compounds. Some products are based on microorganisms and fermented ingredients that can biologically influence the intestinal microbiome.

**Note:**

The products are not purely microalgae-based, but are often combined with bioactive microbial and fermentation-derived ingredients targeting gut health and immune function.



**Company:** EW Nutrition

**Headquarters / Country:** Germany / global operations

**Website:** <https://ew-nutrition.com/animal-nutrition/products/>

**Products / Solutions:**

A range of feed additives for gut health management, antibiotic reduction, and toxin risk management. Some formulations combine bioactive ingredients supporting the microbiome and immune response in pigs and poultry (e.g. the *Activo* product line).

**Note:**

Although the company does not explicitly use the term *microalgae*, some products may contain biologically derived natural components suitable for integrated nutritional programmes.