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Aetiology, immunopathology, histopathology, genetic and nutritional aspects of systemic granulomatosis in meagre (*Argyrosomus regius* (Asso, 1801))-A Review

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ABSTRACT

The meagre (*Argyrosomus regius*) is a teleost species rapidly gaining commercial importance in aquaculture. Under intensive farming conditions, a rising incidence of Systemic Granulomatosis (SG) has been observed in this species. This review comprehensively examines the aetiology, immunopathogenesis, histopathology, nutritional and genetic factors associated with SG. Current scientific evidence strongly suggests that SG is a multifactorial pathology rather than a bacterial infection and is mainly due to non-infectious causes. Studies have shown that molecular positivity for potentially infectious agents such as *Mycobacterium* spp. and *Nocardia* spp. were not confirmed by histopathological and in situ hybridization methods, suggesting that these findings may be related to environmental contamination or non-clinical latent infections. Oxidative stress and inflammatory processes triggered by deficiencies in antioxidant vitamins (C, E and K), n-3 LC-PUFA and imbalanced n-3/n-6 ratios were seen to underlie SG. It has been shown that the incidence of granuloma is significantly reduced with feeds that have an optimal nutrient profile and include balanced live feed applications. Environmental stress factors, especially high stocking density, exacerbate the development of SG by increasing oxidative stress in fish. Therefore, appropriate stocking densities and water quality management play a critical role in disease control. Genetic factors explain individual differences in fish resistance to inflammatory responses and diseases. Research on the meagre genome suggests that adaptive variation in genes associated with the immune system may be involved in susceptibility or resistance to SG. Immunopathologically, SG is a chronic inflammatory response characterized by the transformation of macrophages into epithelioid cells and granuloma formation. Histopathological examinations show the presence of widespread and non-infectious granulomatous lesions in many organs such as heart, liver, kidney and brain. SG is too complex to be explained solely by the presence of pathogens. It should be evaluated together with the genetic and immunological background of the host, dietary lipid profile and susceptibility to environmental stress factors.

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Introduction

The meagre (*Argyrosomus regius*), a teleost species of the Sciaenidae family, is naturally found in the Mediterranean and Atlantic coasts, where it holds significant commercial value (Monfort, 2010). In Türkiye, *Argyrosomus regius* is commonly known as “sarıağız” or “granyöz”. The rapid growth rate of this species, combined with its high fillet yield and physiological structure suitable for intensive farming, has increased its importance in the field of aquaculture, especially in recent years (Zupa et al., 2023). According to FAO FishStatJ data reported by EUMOFA, global meagre production increased from 37,753 tonnes in 2010 to 55,560 tonnes in 2019, representing a 47% rise over the last decade. In Türkiye, meagre (*Argyrosomus regius*) aquaculture production increased from 3,375 tonnes in 2019 to 7,019 tonnes in 2024, corresponding to an approximate 108% increase. Overall, these trends highlight the accelerating commercial relevance of meagre and its strong potential for continued expansion within modern marine aquaculture

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(EUMOFA, 2022; TÜİK, 2025). However, under intensive production conditions, some pathological conditions are observed to occur due to reasons such as stock density, environmental stress factors and nutritional deficiencies, immune dysregulation.

Among these clinical conditions, Systemic Granulomatosis (SG) cases are appear to be the most important problem (Tsertou et al., 2018; Carvalho et al., 2019; 2021; Ruiz et al., 2021; Murgia et al., 2024). SG is a disease characterised by granulomas consisting of well-organised cellular structures which develop due to a chronic inflammatory response. These granulomas are well-organised chronic inflammatory structures consisting of epithelioid macrophages, multinucleated giant cells, fibroblasts and lymphocytes that usually occur in visceral organs in fish (Roberts, 2012; Rajme-Manzur et al., 2021; Murgia et al., 2024)

Ghittino et al. (2004) provided the first rigorous and comprehensive description of SG in meagre, demonstrating that the disease is fundamentally a nutrition-related metabolic condition. Although later literature investigated the potential role of bacterial pathogens—including *Mycobacterium* and *Nocardia*—their detection in granuloma-bearing fish has been inconsistent; despite the presence of granulomas in most cases, there is no evidence of these organisms, and in the studies reporting their occurrence, the limited or unpublished quantitative data do not permit any meaningful evaluation of their prevalence or etiological relevance (Elkesh et al., 2013; Avsever et al., 2014; Tsertou et al., 2018; Gustinelli et al., 2021; Pfalzgraff et al., 2023; Murgia et al., 2024).

These observations indicate that, although these granuloma-inducing pathogens are capable of triggering granulomatous inflammation, they do not represent the primary driver of the highly prevalent and widespread systemic granulomas characteristic of SG in meagre, and their contribution to the overall prevalence of the condition appears to be minimal and limited in scope (Ghittino et al., 2004; Kotzamanis et al., 2018; Carvalho et al., 2019; Ruiz et al., 2019a; Ruiz et al., 2019b; Gustinelli et al., 2021; Pfalzgraff et al., 2023; Murgia et al., 2024; Acosta et al., 2024). Recent academic studies increasingly corroborate the metabolic interpretation advanced by Ghittino et al. (2004), confirming that nutritional deficiencies and metabolic dysregulation remain the dominant factors in SG pathogenesis.

1. Aetiology: Infective and non-infective factors

Various studies have explored the potential involvement of infectious agents in the aetiology of SG in meagre, examining a range of bacterial pathogens through histopathological, bacteriological and molecular approaches.

The first report linking SG lesions to a specific infectious agent was provided by Elkesh et al. (2013), who reported that the aetiology of SG lesions was associated with *Nocardia* infection; macroscopic, histopathological, bacteriological and molecular examinations were performed in 18 meagre. In some of the individuals with SG findings, *Nocardia*-like filamentous structures were identified by histochemical staining, particularly within granuloma structures located in skin and muscle tissues, and the presence of *Nocardia* spp. was molecularly confirmed by PCR in only one muscle tissue sample. However, the fact that the numerical proportion of positive individuals was not specified and the diagnostic methods were applied only to superficial tissues such as skin and muscle limited the etiological evaluation, considering that the disease is predominantly visceral in nature.

A subsequent study by Avsever et al. (2014) described SG pathology in 21 meagre reared under aquaculture conditions in Turkey and reported the first record of *Mycobacterium marinum* detection in this species. PCR analyses indicated *M. marinum* positivity in some individuals, and acid-fast bacterial structures were observed within certain granuloma foci using Ziehl–Neelsen staining. However, numerical data regarding the proportion of positive individuals were not provided, limiting the ability to assess infection prevalence or its potential etiological significance. In addition to these reports, Timur et al. (2015) documented systemic mycobacteriosis caused by *Mycobacterium marinum* in farmed meagre in Türkiye. The investigation was based on six clinically affected fish obtained from a farm, showing signs of loss of appetite, lethargy, emaciation and floating on the water surface. Multifocal granulomas were observed in the liver, spleen and kidney, and Ziehl–Neelsen–positive acid-fast bacilli were detected both in direct smears and histological sections. Bacterial isolation on Lowenstein–Jensen medium yielded photochromogenic colonies, which were subsequently identified as *M. marinum* through hsp65 gene sequencing. This study confirmed that meagre is susceptible to mycobacterial infection; however, because the study's small sample size does not allow for population-level interpretation, its findings offer only limited insight into the epidemiology of the high-prevalence systemic granulomas characteristic of SG.

The study conducted by Tsertou et al. (2018) focused specifically on SG in meagre and evaluated its potential infectious aetiology through an extensive, multi-year investigation. Over a three-year period (2013–2016), fish were sampled from multiple commercial farms and experimental facilities, including inland systems supplied exclusively with borehole seawater as well as sea-cage units. A total of 150 fish diagnosed with SG were subjected to comprehensive macroscopic, histopathological, bacteriological, and molecular analyses. Histological examinations, including Ziehl–Neelsen acid-fast staining, consistently failed to demonstrate acid-fast bacteria within granulomatous lesions. In parallel, PCR-based screening targeting *Mycobacterium* spp. and *Nocardia* spp. revealed no evidence of *Mycobacterium* infection in any of the SG-affected fish, while *Nocardia* seriolae was detected in only a single, geographically confined outbreak, corresponding to a prevalence of 1.3%. Importantly, extended macroscopic and histopathological surveys encompassing approximately 2500 fish examined throughout the study period did not identify any infectious agent consistently associated with granuloma formation, strongly supporting a non-infectious, likely metabolic origin of SG in this species.

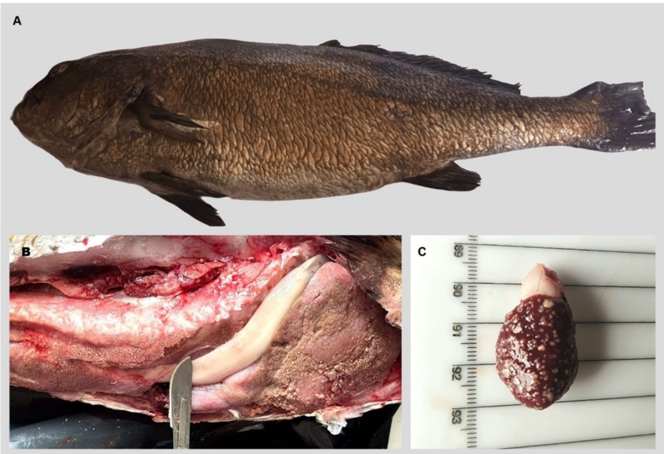

Figure 1. (A) Cultured meagre (*Argyrosomus regius*) reared under aquaculture conditions; (B) macroscopic appearance of the liver surface and abdominal cavity, showing multiple granulomatous lesions affecting the visceral organs; (C) heart of meagre exhibiting multiple granulomatous lesions distributed over the epicardial surface. All photographs are original and were taken by the authors.

Murgia et al. (2024) detected *Mycobacterium* DNA at a rate of 13% in PCR analyses of tissue samples taken from 34 individuals exhibiting SG. However, Ziehl-Neelsen and PAS stains, as well as hybridization assay to confirm the presence of the infectious agent, yielded negative results in the same individuals. Therefore, it was concluded that the positive PCR analysis results could be due to environmental contamination or latent infection.

Gustinelli et al. (2021) further contributed to the etiological evaluation by examining 108 meagre, in which granulomas were observed in 93.5% of individuals. The kidney (93.5%) and liver (39.8%) were the most frequently affected organs, followed by the digestive tract and perivisceral adipose tissue (13.0%), heart (9.3%), spleen (1.9%) and gills (0.9%). Importantly, ZN, PAS, Giemsa and Fite–Faraco stains were negative in all specimens, and no acid-fast bacteria, fungi or parasites were detected within the granulomas. These findings provided further population -level evidence that SG lesions can occur in the absence of identifiable infectious agents, reinforcing the complexity of the condition's aetiology.

Pavloudi et al. (2023) employed a clinical metagenomic approach to identify potential microbial SG. Analyses were conducted using both PEMA (Pipeline for Environmental DNA Metabarcoding Analysis) and Geneious software. Subsequently, the results underwent statistical evaluation via LefSe (Linear Discriminant Analysis Effect Size), PERMANOVA (Permutational Multivariate Analysis of Variance), and nMDS (Non-metric Multidimensional Scaling). In total, 462 OTU (Operational Taxonomic Unit) were identified, but most of these units were found to be common between the groups. Despite the high-sensitivity metagenomic analysis methods used in the study, none of the known granulomatogenic species such as *Mycobacterium*, *Nocardia*, *Francisella*, *Edwardsiella* and *Streptococcus* were found.

In addition to the findings derived from academic studies focusing on meagre, a comparative evaluation of SG cases across different fish species is of particular importance for understanding the aetiological characteristics of this condition. In this context, the large-scale survey conducted throughout Turkey by Avsever et al. (2016), which investigated the relationship between systemic granuloma formation and infectious agents in European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*), while also including other cultured fish species, constitutes an important reference for interspecies comparison. In this study, a total of 1050 harvest-sized (200–400 g) cultured fish were randomly sampled from 35 fish farms, comprising 300 European seabass, 300 gilthead seabream, and 450 trout samples. These samples were evaluated for the presence of *Mycobacterium* spp. using bacteriological, histochemical, and molecular methods. No *Mycobacterium marinum* was detected in fish originating from 34 of the 35 farms examined, and no granulomatous lesions were identified by histopathological examination. In contrast, in a single farm located in the Milas region, all 30 European seabass and 30 gilthead seabream sampled from two separate net cages belonging to the same facility were positive for *M. marinum* (100%), and tubercle-like lesions and systemic granulomas were observed in internal organs at necropsy. These findings demonstrate that granuloma development in European seabass and gilthead seabream does not represent a widespread background pathology, but rather occurs as a consequence of a clearly defined infectious trigger (Avsever et al., 2016).

The infection-dependent granuloma model described in European seabass and gilthead seabream shows a marked divergence when compared with systemic granulomatosis cases reported in meagre. Studies on meagre have consistently reported a high prevalence of granulomatous lesions in the majority of fish groups reared under different production systems; however, specific bacterial pathogens—particularly *Mycobacterium* spp. and *Nocardia* spp.—have been detected only in a limited number of cases. In contrast, while the overall occurrence of granulomas in European seabass and gilthead seabream populations remains low, granulomatous lesions emerge with high consistency almost exclusively in tank, net-cage, or hatchery groups in which *Mycobacterium marinum* is detected (Colorni, 1992; Korun et al., 2005; Ghittino et al., 2004; Elkesh et al., 2013; Avsever et al., 2014; Tsertou et al., 2018; Gustinelli et al., 2021; Pavloudi et al., 2023; Murgia et al., 2024). This contrasting pattern indicates that granulomatous responses in meagre differ markedly from those observed in European seabass and gilthead seabream in terms of prevalence and epidemiological distribution, and therefore that granuloma formation in these species cannot be evaluated within the same aetiological framework. While granuloma development in European seabass and gilthead seabream is strongly associated with classical fish mycobacteriosis, SG in meagre represents an aetiologically distinct condition characterized by high prevalence and species-specific susceptibility. This observation suggests that the immunopathogenesis of SG in meagre is not predominantly driven by direct microbial pathogenesis, as infectious agents appear to contribute only to a limited extent to the high-prevalence SG observed in this species, but is instead shaped by a multifactorial process involving host-specific metabolic and immunological responses to environmental stressors and nutritional imbalances (Ghittino et al., 2004; Kotzamanis et al., 2018; Carvalho et al., 2019; Ruiz et al., 2019a; Ruiz et al., 2019b; Gustinelli et al., 2021; Pfalzgraff et al., 2023; Murgia et al., 2024).

2. Nutrition and environmental factors

With the increase in studies showing the effects of malnutrition in cultured fish, it has become increasingly evident that nutrition plays an important role in the aetiology of SG, and especially feeding strategies and feed composition have a significant effect on the formation of diseases (Ruiz et al., 2019c; Carvalho et al., 2019). Live feeds applied especially during the larval period appear to be critical (Roo et al., 2010; Ruiz et al., 2019c). Ruiz et al. (2019c) reported that granulomas were observed early (on day 20) in larvae fed only enriched rotifer, whereas the incidence of granulomas were significantly lower in the groups fed with *Artemia* from day 12 to day 30. It was determined that larvae fed only rotifers exhibited significantly higher granuloma counts and elevated TBARS (Thiobarbituric Acid Reactive Substances) levels in their tissues compared to the Rotifer + *Artemia* fed groups. The addition of Artemia to the feeding sequence decreased both granuloma incidence and lipid peroxidation.

Antioxidant vitamins have been extensively studied to explain the link between dietary intake and SG in meagre. Most fish species cannot synthesise vitamin C and require fat-soluble antioxidants such as vitamin E. When the effects of vitamins on immune functions are examined; vitamin C increases phagocytic cell functions with its antioxidant capacity, reduces tissue damage by regulating reactive oxygen species (ROS) level and suppresses inflammation by shifting the cytokine balance in favour of IL-10 (Kiron., 2012; Carr and Maggini., 2017). Vitamin E reduces cytokine expression by suppressing NF-κB activity, protects membrane lipids against oxidative damage and promotes controlled immune response (Kiron, 2012; Wu et al., 2024). Vitamin K reduces inflammation-related IL-6 and tnf-α expression and provides immune modulation through the NF-κB pathway (Xie et al., 2024). In the study conducted by Ruiz et al. (2019b), larvae reared in the optimal live feed order (Rotifer+Artemia) until day 30 were fed with micro diets containing different levels of vitamins E and C and increased n-3 ratio by adding krill oil to their diet between days 30–44. It was shown that no granulomas developed in 44-day-old larvae in the highest vitamin group. Similarly, Carvalho et al. (2019) found that providing vitamins E/C/K at higher dietary levels significantly reduced the incidence of granulomas in liver and heart tissues of juvenile meagre compared to the groups receiving lower vitamin levels. Molecular analyses showed that dietary supplementation with vitamins E and C also significantly boosted the expression of vital antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase. Simultaneously, immune response genes such as cox-2 and tnf-α were also strongly induced in fish (Carvalho et al., 2019; Ruiz et al., 2019a). All this suggests that vitamin supplementation triggers a reaction that reduces oxidative damage and suppresses granuloma formation (Ghittino et al., 2004; Carvalho et al., 2019; Ruiz et al., 2019a; Ruiz et al., 2019b).

Beyond the role of antioxidant vitamins, the diet's fatty acid composition has been demonstrated to exert a protective effect against granulomatous inflammations. However, experimental studies conducted by Pfalzgraff et al. (2023) have revealed that meagre lacks sufficient desaturase ("Δ6") and elongase ("Elovl5") enzyme activity to convert C18-PUFAs into long-chain n-3 series polyunsaturated fatty acids (LC-PUFAs) to meet its eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) requirements. Consequently, it is imperative that the fish consumes prey that is abundant in EPA and DHA in order to meet its nutritional requirements. The biochemical basis for the capacity to influence the severity and duration of immune responses is therefore the fatty acid profile of the diet. Specifically, the ratios of n-3 and n-6 polyunsaturated fatty acids regulate the balance of inflammatory eicosanoids. While n-3 fatty acids like EPA and DHA are recognised for their anti-inflammatory properties, n-6 derived arachidonic acid (ARA) contributes to the synthesis of pro-inflammatory mediators such as PGE2 (Calder, 2006; Innes and Calder, 2018; Carvalho et al., 2019). In a study by Bagga et al. (2003), it was shown that diets with high n-6 content significantly increased PGE_2 production and this activated inflammatory gene expression. Therefore, ARA also can play a direct role in the development of pathologies such as granulomatosis by creating a predisposition to chronic inflammation.

In the study by Carvalho et al. (2019), the growth performance, feed efficiency and physiological responses of the fingerlings were evaluated. The fingerlings were fed diets containing 16-17% crude fat and five different n-3 LC-PUFA ratios (0.8, 1.4, 2.0, 2.6 and 3.6%) in a 30-day feeding trial. The total n-3: n-6 fatty acid content of these diets ranged from approximately 0.78 to 2.25. The study's findings indicate that the optimal growth parameters and physiological balance were achieved in groups comprising 2.0-2.6-3.6% n-3 LC-PUFA, exhibiting an n-3: n-6 ratio of approximately 2:1. Conversely, fish fed diets with less than 2.0% n-3 LC-PUFA, particularly those given 0.8% and 1.4%, exhibited a significantly elevated prevalence of non-infectious SG, accompanied by the presence of well-developed granulomatous lesions in the liver tissue. Comprehensive histological, histochemical (special stains), and microbiological analyses confirmed the absence of infectious agents in all affected individuals. Similarly, in another study (Pfalzgraff et al., 2023), a positive correlation was reported between the dietary n-3 LC-PUFA content and growth rate. Granuloma formation in liver tissue was significantly higher in fish fed diets containing less than 0.9% n-3 LC-PUFA, whereas minimal granuloma were observed in the livers of individuals fed with 1.2–1.5% n -3 LC-PUFA. Gustinelli et al. (2021) observed that the incorporation of whole sardine into the diet of cage-reared meagre resulted in a reduction in the severity of liver and intestinal granuloma, in comparison to the pellet-fed group. This finding suggests that the natural nutritional components of sardine, such as high levels of n-3 fatty acids and natural antioxidants, may offer protection against SG. It is therefore vital to include antioxidant supplements, such as vitamins E, C and K, in the formulation of commercial meagre feeds and diets. These should contain optimal levels of 2.0% LC-PUFA and n-3/n-6 (1-2:1) fatty acid ratios. This is widely regarded as being of critical importance in the prevention of SG formation (Carvalho et al., 2019; Ruiz et al., 2019c; Pfalzgraff et al., 2023; Gustinelli et al., 2021).

Furthermore, it has been demonstrated that rearing conditions exert a pivotal role in the regulation of SG severity. It is widely acknowledged that stocking density frequently functions as a substantial stress factor with regard to the health of meagre. As demonstrated in the extant literature, elevated stocking densities are associated with a considerable increase in TBARS levels in reared fish. This finding suggests that lipid peroxidation increases in proportion to density. High stocking densities induce continuous stress in fish, elevating the production of ROS. This surge in ROS can overwhelm natural antioxidant defences, predisposing fish to chronic lesions like SG (Roo et al., 2010; Ruiz et al., 2021). Therefore, maintaining an optimal stocking density approximately 1 L per 100 larvae during the larval period and a stocking density of <5 kg/m^3 during the juvenile period is considered optimal for both growth and the prevention of SG (Roo et al., 2010; Campoverde et al., 2017; Gustinelli et al., 2021; Ruiz et al., 2021).

Tsertou et al. (2020) experimentally evaluated the role of specific dietary components in the development of systemic granulomatosis in meagre. The study demonstrated that dietary vitamin D_3 levels did not exert a significant effect on the onset or severity of systemic granulomatosis. In contrast, fish fed diets with higher phosphorus content exhibited a reduced severity of granulomatous lesions, whereas diets predominantly based on plant protein sources were associated with an increased development and severity of granulomatous pathology. At the same time, Monteiro et al. (2021) conducted a functional feed trial by adding two different seaweeds (*Fucus vesiculosus* and *Nannochloropsis gaditana*) extracts to the feed and it was shown that the supplements improved post-stress cortisol and oxidative parameters and strengthened the immune response in meagre. In addition, Asencio-Alcudia et al. (2019) reported that L-tryptophan added to the diet at 1% ratio to alleviate the stress-induced immune suppression provided immune stability after acute stress by increasing the expression of cox-2, IL-10 and IL-1β genes and decreased cannibalism and agitation by increasing the amount of serotonin. Consequently, the provision of commercial feeds containing optimal levels of LC-PUFA (2.0% with 1-2 n-3: 1 n-6 ratio) and stress-reducing antioxidant agents, such as vitamins E, C and K, in conjunction with enhanced culture conditions (e.g. appropriate fish density, temperature management, minimum handling stress), is of paramount importance in the prevention of SG formation in meagre culture (Roo et al., 2010; Kiron., 2012; Carvalho et al., 2019; Kotzamanis et al., 2018; Pfalzgraff et al., 2019; Ruiz et al., 2019a; Ruiz et al., 2019c; Ruiz et al., 2021; Gustinelli et al., 2021; Asencio-Alcudia et al., 2019; Pfalzgraff et al., 2023; Ruiz et al., 2021; Monteiro et al., 2021).

3. Genetic factors

Although there are still limited data on the genetic factors underlying SG, Campoverde et al. (2019) reported that some key genes of the innate immune system, such as tnf-α, cox-2, myd88, mxp, c3, lyz-c and nod2, were actively expressed from day 8 during the larval development of meagre. The expression levels of key immune-related genes in meagre larvae display developmental variation and undergo marked fluctuations during critical transitions, particularly the shift from live feed to commercial diets. Recent studies on the genome of the meagre species have revealed adaptive differentiation in some genes related to immunity and fat metabolism. In particular, interleukins, interferon receptors, and TLR genes have been shown to be among the rapidly changing loci (Papadogiannis et al., 2023). This new gene resource provides an important basis for investigating quantitative gene loci (QTL) underlying complex phenotypes such as SG. The developed reference genome and identified candidate gene regions have the potential to elucidate the genetic basis of SG and develop resistant strains in the future.

4. Immunopathogenesis and histopathological findings

The immune system of teleost fishes exhibits a structure similar to that of mammals, with innate and adaptive components; among the immune organs in fishes, the thymus, spleen, and pronephros play a central role; the functions of the bone marrow and lymph nodes in mammals are taken over specifically by the anterior kidney (Uribe et al., 2011; Secombes and Ellis, 2012; Mokhtar et al., 2023). Innate immunity forms the first line of defence against pathogens in fish and is divided into physical (skin, gill, intestinal mucosa), humoral (lysozyme, complement, C-type lectins, antimicrobial peptides) and cellular (macrophages, monocytes, neutrophils) components (Magnadóttir, 2006; Uribe et al., 2011; Mokhtar et al., 2023).

Granuloma development is a morphological manifestation of chronic inflammatory processes and begins at the precursor step of the innate immune response (Rajme-Manzur et al., 2021; Weeratunga et al., 2024). In granulomatous lesions, molecules such as the complement system, lysozyme and chemokines are active; cellular effectors such as macrophages, neutrophils and giant cells form the core of the granuloma, while macrophages, in particular, transform into epithelioid cells to forming a peripheral wall that confines persistent pathogens or foreign particles (Rajme-Manzur et al., 2021; Weeratunga et al., 2024).

This process is stringently regulated by proinflammatory cytokines, including tnf-α, Interleukin-1 (IL-1), and Interferon-gamma (IFN-γ) (Zou and Secombes, 2016; Rajme-Manzur et al., 2021; Weeratunga et al., 2024). Specifically, tnf-α and IL-1 facilitate the differentiation of macrophages into epithelioid cells and the formation of multinucleated giant cells, while IFN-γ, secreted by Th1 cells, further reinforces and sustains this granulomatous response (Weeratunga et al., 2024; Rajme-Manzur et al., 2021). These macrophage-derived cells gathered in the centre of the granuloma attract fibroblasts, lymphocytes and possibly B-1 cells, giving the lesion an organised structure (Russo and Mariano, 2010; Zou and Secombes, 2016; Weeratunga et al., 2024). In case of chronic antigenic stimulation, adaptive immunity is activated; T lymphocytes mediate cellular immunity, while B lymphocytes are responsible for the humoral response (Russo and Mariano, 2010; Uribe et al., 2011).

Histologically, granulomas are defined by a regular or irregular distribution of supporting cells such as macrophages, giant cells, lymphocytes and fibrosis differentiated from epithelioid cells around a central necrotic core (not always), and in fish the basic organisation is the same, although this structure shows some differences between species (Roberts, 2012; Rajme-Manzur et al., 2021; Murgia et al., 2024). In meagre, granulomas are more organised, coalescent and encapsulated (Gustinelli et al., 2021; Murgia et al., 2024; Acosta et al., 2024).

In the experimental infection model conducted by Acosta et al. (2024), it was shown that *Nocardia* brasiliensis can cause systemic granulomatous lesions in meagre with high-dose (≥10^6 CFU/ml) exposure. However, since clinical findings and histopathological prevalence were quite low in lower-dose exposure models mimicking environmental contamination and the presence of bacteria was not detected in some granuloma structures, it was emphasised that SG is not limited to infectious agents and that non-infectious factors such as nutritional disorders, environmental stress and immune dysregulation may also play a role in the aetiology. In this study, granulomas were classified into five distinct structural types based on their histological morphology, which were described in detail. In the study by Acosta et al, (2024), fluorescent signals specific for *Nocardia* antigens were detected in some granulomas, while the presence of bacterial antigens could not be detected in other structures. This distinction shows that both infective and non-infective granulomas can coexist in the same histological tissue (Figure 2).

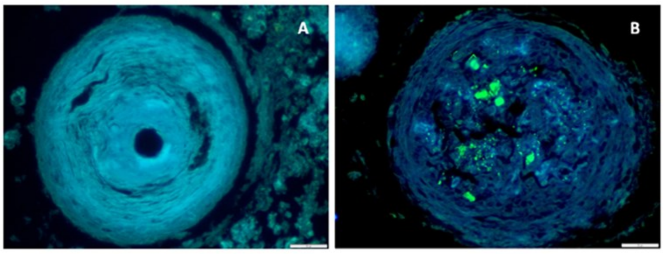

Figure 2. [Indirect immunofluorescence staining of *Nocardia* spp. in granulomatous liver tissue of *Argyrosomus regius*.] (A) Non-infective granuloma. (B) *Nocardia*l aggregates visible in bright green fluorescence in a positive granuloma. Magnification: 40×; scale bar = 20 µm. Reproduced as originally published in Acosta et al. (2024), Aquaculture, 581, 740458. Licensed under the Creative Commons Attribution 4.0 International (CC BY 4.0).

Carvalho et al. (2019) divided the observed granulomas into two basic morphological types, Early stage granulomas and Advanced stage granulomas. Gram staining, ZN and PAS staining were negative in all samples. No pathogenic microorganisms were grown in bacteriological cultures. This classification shows a clear morphological overlap with the granuloma structures described by Acosta et al. (2024) and Gustinelli et al. (2021). Importantly, the findings of Carvalho et al. (2019) demonstrate that similar granuloma maturation stages can develop in the absence of detectable infectious agents, as no pathogens were identified by histochemical staining or bacteriological culture, supporting the concept that comparable granulomatous architectures may arise independently of confirmed infectious etiology.

Murgia et al. (2024) reported multifocal granulomatous lesions in 91% of 34 adult meagre. Histologically, granulomas were characterised by a necrotic core composed of eosinophilic granular material and cellular debris, surrounded by multilayered epithelioid cells and an outer fibroblastic zone, consistent with classical granulomatous inflammation. Macroscopically, severely affected fish exhibited calcified kidneys and livers, extensive granuloma coverage of the heart, and multiple granulomas in soft tissues. Despite this widespread pathology, no bacteria, fungi, or parasites were detected by Ziehl–Neelsen, PAS, or Giemsa staining. At the molecular level, PCR analyses detected *Mycobacterium* DNA in a limited proportion of samples, showing high similarity to the hsp65 gene of *Mycobacterium chelonae*; however, in situ hybridisation assays were consistently negative. These findings indicate that molecular detection alone does not provide sufficient evidence of an active infectious aetiology and highlight the necessity of integrating histopathological and molecular approaches when interpreting granulomatous lesions.

Therefore, there is a clear need for the development of new diagnostic methods specifically aimed at distinguishing infective and non-infective granulomas, and for further research to be conducted in this area.

Conclusions

SG seen in meagre is a complex pathology that causes significant economic losses in aquaculture and whose aetiology has not been fully elucidated. Current data reveal that SG is not directly related to an infectious agent, but rather is shaped by the interaction of multiple factors such as nutrition, environmental stress and genetic predisposition (Carvalho et al., 2019; Pfalzgraff et al., 2023; Murgia et al., 2024).

Overall, the accumulated evidence from histopathology, experimental models, and recent metagenomic studies converges on a common conclusion: infectious agents, although capable of inducing granulomatous responses under specific conditions, contribute only marginally to the overall burden of SG in meagre. The high prevalence and systemic distribution of granulomas are more consistently explained by nutritional imbalances and metabolic dysregulation, in line with the original hypothesis proposed by Ghittino et al. (2004). (Tsertou et al., 2018; Ruiz et al., 2019a; Ruiz et al., 2021; Gustinelli et al., 2021; Monteiro et al., 2021; Pfalzgraff et al., 2023). In addition to live feed transition strategies in the larval stage, the insufficiency of larval and juvenile diets in terms of n-3 LC-PUFA and antioxidant vitamins (C, E, K) may trigger granuloma formation by increasing oxidative stress levels (Carvalho et al., 2019; Ruiz et al., 2019a; Pfalzgraff et al., 2023). These findings clearly demonstrate the necessity of optimised commercial feed formulations to meet the rapid growth performance of meagre.

Environmental factors also play a critical role in SG development. High stocking density, for instance, results in elevated TBARS concentrations and a build up of ROS. This excess ROS can overwhelm the antioxidant defences of fish, leading to chronic inflammation (Ruiz et al. 2021). Temperature fluctuations, poor water quality, and stress from handling are also among the factors that exacerbate this process. The use of stress-reducing feed additives (e.g. L-tryptophan, algal extracts) offers potential benefits in controlling this pathological response (Monteiro et al., 2021; Gustinelli et al., 2021).

Genetic factors are important in determining the susceptibility of fish to infectious and non-infectious diseases. In multifactorial diseases such as SG, genetic predisposition can significantly affect individual resistance levels and immune response capacity. In the case of meagre, the observation that the expression levels of proinflammatory genes (e.g. cox-2, tnf-α) increased in individuals fed with high vitamin C and E suggests that the granulomatous response may have a genetic component (Ruiz et al., 2019a; Carvalho et al., 2019). The genomic analyses conducted by Papadogiannis et al. (2023) revealed adaptive differentiation in some gene regions related to immunity and lipid metabolism; in particular, interleukins, interferon receptors and Toll-like receptor (TLR) genes were shown to be among the rapidly evolving loci. These findings indicate that the development of lines with increased genetic resistance to SG through selection offers an important strategic opportunity for sustainable aquaculture practices.

In terms of immunopathogenesis, granuloma formation appears to be a prolonged and inadequate response of the innate immune system. The transformation of macrophages into epithelioid cells, giant cell formation and cytokine network (tnf-α, IL-1, IFN-γ, IL-6, IL-12) reveal the complex structure and chronicity mechanism of granuloma (Zou and Secombes, 2016; Rajme-Manzur et al., 2021; Gustinelli et al., 2021; Murgia et al., 2024). Histopathological findings confirm the presence of widespread granulomatous lesions in various organs such as heart, liver, kidney and brain (Murgia et al., 2024). This prevalence and the fact that the lesions do not contain infectious agents indicate the systemic nature of the disease and that nutritional-environmental factors can affect the entire body (Pavloudi et al., 2023; Murgia et al., 2024).

In conclusion, pathogen-focused approaches alone are insufficient for the prevention and control of SG. Optimising nutritional strategies, reducing environmental stress and selecting resistant individuals through genetic selection provide a holistic roadmap to combat the disease. Today, producers can control SG to a large extent with high quality fish oil, antioxidant vitamin supplementation and balanced live feed transitions. In order to both protect fish health and ensure sustainable aquaculture production, future research should focus on elucidating the molecular and immunological mechanisms of SG in more depth and developing new strategies for more effective management and prevention of the disease.

References

Acosta, F., Vega, B., Monzón-Atienza, L., Superio, J., Torrecillas, S., Gómez-Mercader, A., & Galindo-Villegas, J. (2024). Phylogenetic reconstruction, histopathological characterization, and virulence determination of a novel fish pathogen, *Nocardia brasiliensis*. *Aquaculture, 581*, 740458.

Asencio-Alcudia, G., Andree, K. B., Giraldez, I., Tovar-Ramirez, D., Alvarez-González, A., Herrera, M., & Gisbert, E. (2019). Stressors due to handling impair gut immunity in meagre (*Argyrosomus regius*): the compensatory role of dietary L-Tryptophan. *Frontiers in Physiology, 10*, 547.

Avsever, M. L., Çavuşoğlu, C., Günen, M. Z., Yazıcıoğlu, Ö., Eskiizmirliler, S., Didinen, B. I., & Özden, M. (2014). The first report of *Mycobacterium marinum* isolated from cultured meagre, Argyrosomus *regius*. *Bulletin of the European Association of Fish Pathologists 34*(4).

Avsever, M. L., Çavuşoğlu, C., Eskiizmirliler, S., Türe, M., Korun, J., & Çamkerten, I. (2016). First isolation of *Mycobacterium marinum* from sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus auratus*) cultured in Turkey. Bulletin of the European Association of Fish Pathologists, 36, 193.

Bagga, D., Wang, L., Farias-Eisner, R., Glaspy, J. A., & Reddy, S. T. (2003). Differential effects of prostaglandin derived from ω-6 and ω-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. *Proceedings of the National Academy of Sciences, 100*(4), 1751-1756.

Calder, P. C. (2006). Polyunsaturated fatty acids and inflammation. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 75*(3), 197-202.

Campoverde, C., Rodriguez, C., Perez, J., Gisbert, E., & Estévez, A. (2017). Early weaning in meagre *Argyrosomus regius*: Effects on growth, survival, digestion and skeletal deformities. *Aquaculture Research, 48*(10), 5289-5299.

Campoverde, C., Milne, D. J., Secombes, C. J., Estévez, A., Gisbert, E., & Andree, K. B. (2019). Gene expression analysis of the innate immune system during early rearing and weaning of meagre (*Argyrosomus regius*). *Fish & Shellfish Immunology, 94*, 819-832.

Carr, A. C., & Maggini, S. (2017). Vitamin C and immune function. *Nutrients, 9*(11), 1211.

Carvalho, M., Castro, P., Montero, D., Peres, H., Acosta, F., Fontanillas, R., & Izquierdo, M. (2019). Essential fatty acid deficiency increases hepatic non-infectious granulomatosis incidence in meagre (*Argyrosomus regius*, Asso, 1801) fingerlings. *Aquaculture, 505*, 393 -404.

Colorni, A. (1992). A systemic mycobacteriosis in the European sea bass *Dicentrarchus labrax* cultured in Eilat (Red Sea). *Israeli Journal of Aquaculture – Bamidgeh, 44*, 75.

Elkesh, A., Kantham, K. P. L., Shinn, A. P., Crumlish, M., & Richards, R. H. (2013). Systemic nocardiosis in a Mediterranean population of cultured meagre, *Argyrosomus regius* Asso (*Perciformes: Sciaenidae*). *Journal of Fish Diseases, 36*(2), 141-149.

EUMOFA. (2022). Meagre in the EU. European Market Observatory for Fisheries and Aquaculture Products. Luxembourg: *Publications Office of the European Union*, 2022.

Ghittino, C., Manuali, E., Latini, M., Agnetti, F., Rogato, F., Agonigi, R., ... & Prearo, M. (2004). Case of systemic granulomatosis in meagre (*Argyrosomus regius*) and comparison with the histological features present in gilthead seabream. *Ittiopatologia, 1*, 59-67.

Gustinelli, A., Čolak, S., Quaglio, F., Sirri, R., Kolega, M., Caffara, M., & Fioravanti, M. L. (2021). Histological assessment of systemic granulomatosis progression in meagre *Argyrosomus regius* during cage ongrowing phase. *Diseases of Aquatic Organisms, 145*, 165-172.

Innes, J. K., & Calder, P. C. (2018). Omega-6 fatty acids and inflammation. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 132*, 41-48.

Kiron, V. (2012). Fish immune system and its nutritional modulation for preventive health care. *Animal Feed Science and Technology, 173*(1-2), 111-133.

Korun, J., Olgac, V., Akgun-Dar, K., Colorni, A., & Diamant, A. (2005). Mycobacteriosis in European sea bass, l., cultured in Turkey. *Israeli Journal of Aquaculture-Bamidgeh, 57*.

Kotzamanis, Y., Kouroupakis, E., Ilia, V., Haralabous, J., Papaioannou, N., Papanna, K., ... & Gisbert, E. (2018). Effects of high-level fishmeal replacement by plant proteins supplemented with different levels of lysine on growth performance and incidence of systemic noninfectious granulomatosis in meagre (*Argyrosomus regius*). *Aquaculture Nutrition, 24*(6), 1738-1751

Magnadóttir, B. (2006). Innate immunity of fish (overview). *Fish & Shellfish Immunology, 20*(2), 137-151.

Mokhtar, D. M., Zaccone, G., Alesci, A., Kuciel, M., Hussein, M. T., & Sayed, R. K. A. (2023). Main Components of Fish Immunity: An Overview of the Fish Immune System. *Fishes, 8*(2), 9

Monfort, M.C. Present market situation and prospects of meagre (*Argyrosomus regius*), as an emerging species in Mediterranean aquaculture. In Studies and Reviews, *General Fisheries Commission for the Mediterranean* No. 89; FAO: Roma, Italy, (2010); p. 28.

Monteiro, M., Sousa, C., Coutinho, F., Castro, C., Fontinha, F., Guerreiro, I. & Couto, A. (2021). Functional feeds to tackle meagre (*Argyrosomus regius*) stress: Physiological responses under acute stressful handling conditions. *Marine Drugs, 19*(11), 598.

Murgia, C., Cubeddu, T., Burrai, G. P., Alberti, A., Bertolotti, L., Colitti, B., Prearo, M., Pastorino, P., Esposito, G., Mandrioli, L., Barbera, G., Sanna, M. A., Polinas, M., Soto, E., & Antuofermo, E. (2024). Systemic Granulomatosis in the Meagre *Argyrosomus regius*: Fishing for a Plausible Etiology. *Veterinary Sciences, 11*(12), 597.

Papadogiannis, V., Manousaki, T., Nousias, O., Tsakogiannis, A., Kristoffersen, J. B., Mylonas, C. C. & Tsigenopoulos, C. S. (2023). Chromosome genome assembly for the meagre, *Argyrosomus regius*, reveals species adaptations and sciaenid sex-related locus evolution. *Frontiers in Genetics, 13*, 1081760.

Pavloudi, C., Tsertou, M. I., Antonopoulou, E., & Katharios, P. (2023). Investigation of systemic granulomatosis in cultured meagre, *Argyrosomus regius*, using clinical metagenomics. *Aquaculture, 567*, 739249.

Pfalzgraff, T., Borges, P., Robaina, L., Kaushik, S., & Izquierdo, M. (2023). Essential fatty acid requirement of juvenile meagre (*Argyrosomus regius*). *Aquaculture, 572*, 739532.

Rajme-Manzur, D., Gollas-Galván, T., Vargas-Albores, F., Martínez-Porchas, M., Hernández-Oñate, M. Á., & Hernández-López, J. (2021). Granulomatous bacterial diseases in fish: An overview of the host's immune response. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 261*, 111058.

Roberts, R. J. (2012). *The bacteriology of teleosts, in Roberts, R. J (Ed). Fish pathology 4th ed*. (pp.339-383) John Wiley & Sons, US: New Jersey

Roo, J., Hernández-Cruz, C. M., Borrero, C., Schuchardt, D., & Fernández-Palacios, H. (2010). Effect of larval density and feeding sequence on meagre (*Argyrosomus regius*; Asso, 1801) larval rearing. *Aquaculture, 302*(1-2), 82-88.

Ruiz, M. A., Betancor, M. B., Robaina, L., Montero, D., Hernández-Cruz, C. M., Izquierdo, M. S. & Caballero, M. J. (2019a). Dietary combination of vitamin E, C and K affects growth, antioxidant activity, and the incidence of systemic granulomatosis in meagre (*Argyrosomus regius*). *Aquaculture, 498*, 606-620.

Ruiz, M. A., Hernández-Cruz, C. M., Caballero, M. J., Fernandez-Palacios, H., Saleh, R., Izquierdo, M. S., & Betancor, M. B. (2019b). Appearance of systemic granulomatosis is modulated by the dietary supplementation of vitamin E and C in meagre (*Argyrosomus regius*) larvae fed inert microdiets. *Aquaculture, 506*, 139-147.

Ruiz García, M. Á., Hernández-Cruz, C. M., Caballero, M. J., Fernández-Palacios, H., Saleh, R., Izquierdo, M., & Betancor Quintana, M. B. (2019c). Incidence of systemic granulomatosis is modulated by the feeding sequence and type of enrichment in meagre (*Argyrosomus regius*) larvae. *Aquaculture Research, 50*(1), 284-295.

Ruiz, M. Á., Betancor, M. B., Montero, D., Caballero, M. J., Hernández-Cruz, C. M., Rosenlund, G. & Izquierdo, M. S. (2021). The effect of fish stocking density and dietary supplementation of vitamin C and micronutrients (Mn, Zn and Se) on the development of systemic granulomatosis in juvenile meagre (*Argyrosomus regius*). *Aquaculture Research, 52*(11), 5703-5718.

Russo, R. T., & Mariano, M. (2010). B-1 cell protective role in murine primary *Mycobacterium bovis bacillus Calmette-Guerin* infection. *Immunobiology, 215(*12), 1005-1014.

Secombes, C. J. & Ellis, A. E. (2012). The immunology of teleosts. in Roberts, R. J (Ed). Fish pathology 4th ed. (pp.339-383) John Wiley & Sons, US: New Jersey

TÜİK, (2025). Su Ürünleri İstatistikleri https://biruni.tuik.gov.tr/medas/?kn=97&locale=tr (Erişim tarihi: 12.12.2025).

Tsertou, M. I., Smyrli, M., Kokkari, C., Antonopoulou, E. & Katharios, P. (2018). The aetiology of systemic granulomatosis in meagre (*Argyrosomus regius*): The "*Nocardia*" hypothesis. *Aquaculture Reports, 12*, 5-11.

Tsertou, M. I., Chatzifotis, S., Fontanillas, R., Cotou, E., Fountoulaki, E., Antonopoulou, E., & Katharios, P. (2020). The effect of dietary vitamin D3, minerals (Ca, P) and plant-protein sources in the development of systemic granulomatosis in meagre (*Argyrosomus regius*, Asso, 1801). *Aquaculture, 521*, 735052.

Timur, G., Ürkü, Ç., Çanak, Ö., Genç, E. G., & Erturan, Z. (2015). Systemic Mycobacteriosis Caused by *Mycobacterium marinum* in Farmed Meagre (*Argyrosomus regius*), in Turkey. *Israeli Journal of Aquaculture-Bamidgeh*, 67.

Uribe, C., Folch, H., Enríquez, R. & Moran, G. J. V. M. (2011). Innate and adaptive immunity in teleost fish: a review. *Veterinární Medicína, 56*(10), 486-503.

Weeratunga, P., Moller, D. R. & Ho, L. P. (2024). Immune mechanisms of granuloma formation in sarcoidosis and tuberculosis. *The Journal of Clinical Investigation, 134*(1).

Wu, Q., Luo, Y., Lu, H., Xie, T., Hu, Z., Chu, Z. & Luo, F. (2024). The potential role of vitamin E and the mechanism in the prevention and treatment of inflammatory bowel disease. *Foods, 13*(6), 898.

Xie, Y., Li, S., Wu, D., Wang, Y., Chen, J., Duan, L. & Li, Y. (2024). Vitamin K: infection, inflammation, and auto-immunity. *Journal of Inflammation Research, 1147-*1160.

Zou, J., & Secombes, C. J. (2016). The function of fish cytokines. *Biology, 5*(2), 23.

Zupa, R., Hala, E., Ventriglia, G., Pousis, C., Passantino, L., Quaranta, A. & De Virgilio, C. (2023). Reproductive maturation of meagre *Argyrosomus regius* (Asso, 1801) reared in floating cages. *Animals, 13*(2), 223.