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Title

Circular economy in aquaculture: formulation of feed from agricultural by products and wasts to improuve growth, metabolism and immunity system of Red Tilapia.

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I dedicate this modest work to…

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الملخص:

مع تزايد الحاجة إلى تربية مائية مستدامة، تستكشف هذه الدراسة إمكانية استخدام نواتج ثانوية زراعية صناعية محلية هدفت الدراسة إلى تقييم آثار أربعة نظم .(Oreochromis sp.) المصدر كبدائل للأعلاف التجارية لأسماك البلطي الأحمر غذائية تجريبية — ثلاثة معتمدة على مكونات طبيعية (الخروب، بقايا عصر العنب، السبيرولينا والمورينجا) ونظام تجاري واحد (ONAB) — على أداء النمو، والمعايير الدموية، والعلامات الكيميائية الحيوية، والاستجابة المناعية الفطرية.

أظهر النظام الغذائي المعتمد على الخروب (النظام 1) تحسناً ملحوظاً في النمو (زيادة الوزن 192 جم، متوسط الزيادة اليومية 5.65 جم/يوم)، وتحسناً في معدل تحويل العلف (FCR = 2.660.38 ؛ كفاءة العلف) بالإضافة إلى ارتفاع في ، مؤشرات دموية رئيسية مثل البروتين البلازمي (45.52 جم/لتر)، وسكر الدم (67.38 ملغ/ديسيلتر)، ونشاط الليزوزيم (6.11 وحدة/مل). في المقابل، سجل النظام المعتمد على بقايا عصر العنب (النظام 2) أقل أداء، يُعزى ذلك إلى محتواه العالي من العفص والألياف الذي قلل من الهضم وامتصاص المغذيات. بينما قدم النظام المكون من السبيرولينا والمورينجا (النظام 3) فوائد متوسطة.

ينبغي أن تركز الأبحاث المستقبلية على التحليل النسيجي، ودراسة تكوين الميكروبيوم المعوي، وتعبير الجينات المناعية، ودراسة الجدوى الاقتصادية في ظروف شبه صناعية للتحقق من تطبيق هذه الأعلاف البديلة عملياً.

الكلمات المفتاحية:

البلطي الأحمر (Oreochromis sp.)، العلف الوظيفي، نواتج ثانوية زراعية صناعية، الخروب، السبيرولينا، بقايا عصر العنب، المورينجا، أداء النمو، كفاءة العلف، المعايير الدموية، العلامات الكيميائية الحيوية، نشاط الليزوزيم، تربية مائية مستدامة

Abstract:

With the growing need for sustainable aquaculture, this study explores the use of locally available agro-industrial by-products as alternatives to commercial feeds for red tilapia (*Oreochromis sp.).* The objective was to assess the effects of four experimental diets—three based on natural ingredients (carob, grape pomace, spirulina, moringa) and one commercial feed (ONAB) on growth performance, hematological parameters, biochemical markers, and innate immunity. The carob-based diet (Diet 1) significantly enhanced growth (WG = 192 g, DWG = 5.65 g/day), improved feed conversion (FCR = 2.66; FE = 0.38), and elevated key blood markers such as plasma protein (45.52 g/L), glucose (67.38 mg/dL), and lysozyme activity (6.11 U/mL).

In contrast, the grape pomace-based diet (Diet 2) showed the lowest performance, likely due to its high tannin and fiber content, which reduced digestibility and nutrient absorption. The spirulina-moringa-based diet (Diet 3) offered intermediate benefits. Future research should focus on histological analysis, gut microbiota profiling, gene expression of immune markers, and economic feasibility under semi-industrial conditions to validate the practical application of these alternative feeds.

Keywords: Red Tilapia (*Oreochromis sp.*), functional feed, agro-industrial by-products, carob, spirulina, grape pomace, moringa, growth performance, feed efficiency, hematological parameters, biochemical markers, lysozyme activity, sustainable aquaculture.

Résumé :

Face au besoin croissant d'une aquaculture durable, cette étude explore l'utilisation de coproduits agro-industriels locaux comme alternatives aux aliments commerciaux pour le tilapia rouge (*Oreochromis sp.*). L'objectif était d'évaluer les effets de quatre régimes expérimentaux : trois à base d'ingrédients naturels (caroube, marcs de raisin, spiruline et moringa) et un régime commercial (ONAB), sur la croissance, les paramètres hématologiques, les marqueurs biochimiques et l'immunité innée.

Le régime à base de caroube (Régime 1) a significativement amélioré la croissance (gain en poids = 192 g, gain moyen journalier = 5,65 g/j), optimisé la conversion alimentaire (IC = 2,66 ; efficacité alimentaire = 0,38) et augmenté des indicateurs sanguins clés tels que les protéines plasmatiques (45,52 g/L), la glycémie (67,38 mg/dL) et l'activité de la lysozyme (6,11 U/mL). En revanche, le régime à base de marcs de raisin (Régime 2) a présenté les performances les plus faibles, vraisemblablement en raison de sa teneur élevée en tanins et en fibres réduisant la digestibilité et l'absorption des nutriments. Le régime spiruline–moringa (Régime 3) a offert des bénéfices intermédiaires.

Les recherches futures devraient se concentrer sur l'analyse histologique, le profilage du microbiote intestinal, l'expression génique des marqueurs immunitaires et la faisabilité économique en conditions semi-industrielles afin de valider l'application pratique de ces aliments alternatifs.

Mots-clés :

Tilapia rouge (*Oreochromis sp.)*, alimentation fonctionnelle, coproduits agro-industriels, caroube, spiruline, marcs de raisin, moringa, performance de croissance, efficacité alimentaire, paramètres hématologiques, marqueurs biochimiques, activité de la lysozyme, aquaculture durable.

Abbreviation list:

ALT: alanine aminotransferase.

BWG : Body weight gain.

CMV: Complements menirals &vitamins.

D1: diet 1 (carob diet).

D2: diet 2 (pomace gaps).

D3: diet 3(spirullina diet).

Diet 4: ONAB

DWG : Daily weight gain.

FAO: food and agriculture organization.

FCR :Feed conversion ratio.

FE: feed efficiency.

GLU: glucose.

Hb :Hemoglobin.

HB: hemoglin.

HCT: hematocrit.

Ht : Hematocrit.

PG: pomace gaps.

PH: potentiel hydrogene.

RBC : Red blood cell.

SR: survival rate.

SRP :Survival rate percentage.

TC: total cholesterol.

TG: Triglycerides.

TP: total protein.

WBC :White blood cell .

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Introduction

Introduction

In a world with a growing demand for clean water and healthy food, the economy in a linear model is no longer adequate but circular economy can help reduce the use of incresesingly scarce resources reduce waste production and limit energy consumption (Sergio. Z, 2023).

Aquaculture has emerged as one of the fastest-growing food production sectors worldwide, offering a sustainable to overexploited marine fisheries and playing a crucial role in global food security(Garlock et *al*., 2022). However, the rapid intensification of aquaculture practices has led to several challenges, including increased feed costs, evvironmental impacts from waste(Kumar et *al*., 2023), and health issues in farmed fish such as Red Tilapia (*Oreochromis spp)*, a species widely cultivated for its economic value and adaptability(Ekasari et *al*., 2022).

Tilapia is currently the world's most widely farmed fish species, with production reaching 6.3 million metric tons in 2023 and generating more than $12 billion in value (FAO, 2023),it plays an increasingly important role in human nutrition, particularly in regions where fish products are scarce. Its flesh is highly valued for being rich in high-quality proteins (about 18–20%), essential amino acids, beneficial fatty acids (such as omega-3 and omega-6), vitamins (B12, D), and minerals like phosphorus (Correia et *a*l., 2019).

In arid and semi-arid regions, where nutritional deficiencies in animal proteins and fishery products are common, tilapia farming offers a sustainable solution to meet the growing demand for nutritious food (Tahir et *al.,* 2024).However, tilapia aquaculture faces challenges such as the high cost of feed ingredients and shortages due to import difficulties(Chary et *al.*, 2024).

In this context, the concept of the circular economy presents an innovative and sustainable approach to aquaculture. By reusing, recycling, and reducing organic waste, circular systems aim to optimize resource utilization and minimize environmental burdens(Kusumowardani & Tjahjono., 2020).

Agriculture and agro-industrial by-products, often considered wast, can be repurposed into valuable feed ingredients(Strazza et *a*l., 2015). These alternative feed sources may not only reduce dependency on commercial feeds but also improve fish growth, health, and product quality (Laso et *al.*, 2017).

This study investigates the potential of integrating plant-based additives and local agro-waste products into Red Tilapia feed formulations as part of a circular economy model.

The objective is to asses' thier impact on growth performance, hematological and biochemical responses, and the nutritional composition of the fish flesh. By analyzing and comparing fourdifferent diets, including commercial feed, plant-enriched diets, and locally produced feed.

The resaerch aims to identify sustainable solutions that enhance both productivity and environmental responsibility in freshwater aquaculture.

Through this work, we hope to contribute to the development of cost-effective, health promoting, and eco-friendly feeding strategies for small and medium scale fish farms

General view about Red Tilapia

1. Red tilapia (*Oreochromis spp*.)

Red hybrid Tilapia (*Oreochromis spp*) is an omnivorous species and highly preferred cultured fish (FAO, 2022). It is a valuable hybrid species used in worldwide aquaculture, known for its fast growth rate, tolerance to variable environmental conditions, and attractive red coloration, which is preferred by consumers (Ahmad et al., 2023). This hybrid is often developed through selective breeding and crossbreeding among species such as *Oreochromis mossambicu*s, *Oreochromis niloticu*s, and *Oreochromis aureu*s (FAO, 2020). It thrives in both fresh and brackish water environments, making it suitable for cultivation in aquaculture systems globally (Siti N, 2021).

Figure 1. Red tilapia fish.

1.2 Taxonomic hierarchy of red tilapia(according to NCBI)

- **Superkingdom** : eukaryota.
- **Superorder** : Cichlomorphae Kingdom : metazoa.
- **Phylum:** Chordata. (Possess a notochord, dorsal nerve cord, and pharyngeal slits at some stage of development)
- **Subphylum** : Craniata.
- **Infraclass** : Teleostei.
- **Cohort** : Euteleosteomorpha.
- **Order**: Cichliformes. (A diverse order of freshwater fishes, including cichlids).
- **Family:** Cichlidae. (Cichlids, known for their diversity and adaptability).
- **Subfamily :** Pseudocrenilabrinae.
- **Tribe** : Oreochromini.
- **Genus** : *Oreochromis*.

- **Species**: *Oreochromis spp,* (Hybrid) (Red tilapia is typically a hybrid of *Oreochromis mossambicus, Oreochromis niloticus, and Oreochromis aureus*).

1.3Biological and ecological characteristics of *oreochromis spp:*

Red Tilapia (*Oreochromis spp*) is known for its resilience and adaptability to environmental changes, making it an excellent choice for aquaculture systems (A.Rahman, 2020). Its biological and ecological characteristics are crucial for creating sustainable farming conditions (Nadirah, 2017).

1.3.1 Temperature

Its best development be in water temperatures between 26–32°C (Kamal, 2010).

1.3.2 Salinity

Can livein both freshwater and brackish water, thanks to its ability to handle different salt levels,reaching up to 25-30 ppt (Heba,2024).

1.3.3 Dissolved oxygen

The survival and growth of red Tilapia rely heavily on the availability of dissolved oxygen.They thrive best in environments with at least 5 mg/L of (Muin, 2024). Levels below 3 mg/L cause stress,reduce feeding, and increase death rates(El-Sherif, 2008). To maintain suitable DO levels in aquaculture, effective water circulation and proper aeration systems are essential ,as advised (Boyd, 2020).

1.3.4 pH

The best and ideal pH range for Red Tilapia is 7.0 to 8.5, with an acceptable range of 6.5 to 9.0 (Sallam G, 2024). Levels outside 6.0 to 9.5 can harm growth, reduce feed efficiency, and increase disease risk. Stale pH is essential for their health and productivity (Stickney, 2006).

1.3.5 Nitrogen compounds

In tilapia aquaculture, nitrogen compounds like ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) are key indicators of water quality. Red Tilapia can handle moderate levels, but high concentrations are harmful (Boyd, 2020).

- Ammonia: Should stay below 0.5 mg/L to avoid gill damage and growth issue (Tilak, K. S.2006).
- Nitrite: The safe limit is 0.1mg/L, as higher levels can interfere with oxygen transport in the blood (Kroupova, 2008).
- Nitrate: Should be under 50mg/L; consistently high levels can impact growth and immune health (Boyd, 2014).

1.4 Morphological features of *oreochromis spp*:

1.4.1. Morphology

Red Tilapia has a body shape similar to other cichlid fish, being flat from side to side (Ciezarek 2024). Its pectoral fins, located on the sides, help it stay stable and move easily in the .. The fish usually has a red or pink color, but this can change depending on its genes, diet and environment(Fang et *al*., 2021).Some may also have dark spots or stripes(Nguyen et *al*., 2017).Its body is covered with smooth, round scales that reduce friction while swimming(Decano et *al.,*2020).Red tilapia has including a long dorsal fin on its back with both hard and soft rays a tail fin that helps it move forward, and an anal fin that differs in shape between males and females(Lopez et *al*., 2019).its mouth faces forward and can stretch a little, allowing it to eat plankton, waste and artificial food .its eyes are on the sides of its head, helping it see predators and find food(El-Sayed, 2006).

1.4.2 Anatomy

The internal anatomy of Red Tilapia is adapted to its omnivorous feeding habits and aquatic lifestyle:

Figure 2. Red Tilapia anatomy (2018).

1.4.2.1. Digestive system

It possesses small teeth in their mouth that facilitates the handling and grinding of feed (Stickney, 2006). Their digestive system includes a J-shape stomach, which plays a key role in breaking down ingested material (FAO, 2020). Following the stomach, along and coiled intestine allows for effective absorption of nutrients, a feature well suited to their omnivorous diet (EL-Sayed, 2006).

1.4.2.2 Respiratory system:

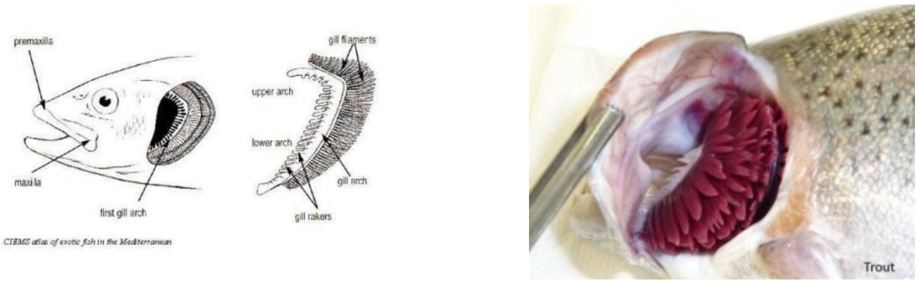

Figure 3. Respiratory system of Red Tilapia.

Equipped with four pairs of gills supported by gill arches, this species ensures efficiency oxygen exchange in aquatic environments (Trewavas,1983). Its swim bladder, agas-filled chambre, plays a crucial role in regulating buoyancy and maintaining stability across varying water depths (Stickney,2006).

1.4.2.3 Reproductive system

Males: Fish have a single urogenital passageway, which often includes an altered anal fin structure serving as a sperm transfer tool (El-Sayed, 2006).

Females: The female fish maintains separate genital and urinary openings for egg carrying functions in combination with the mouth-brooding behavior observed in (FAO .2023).

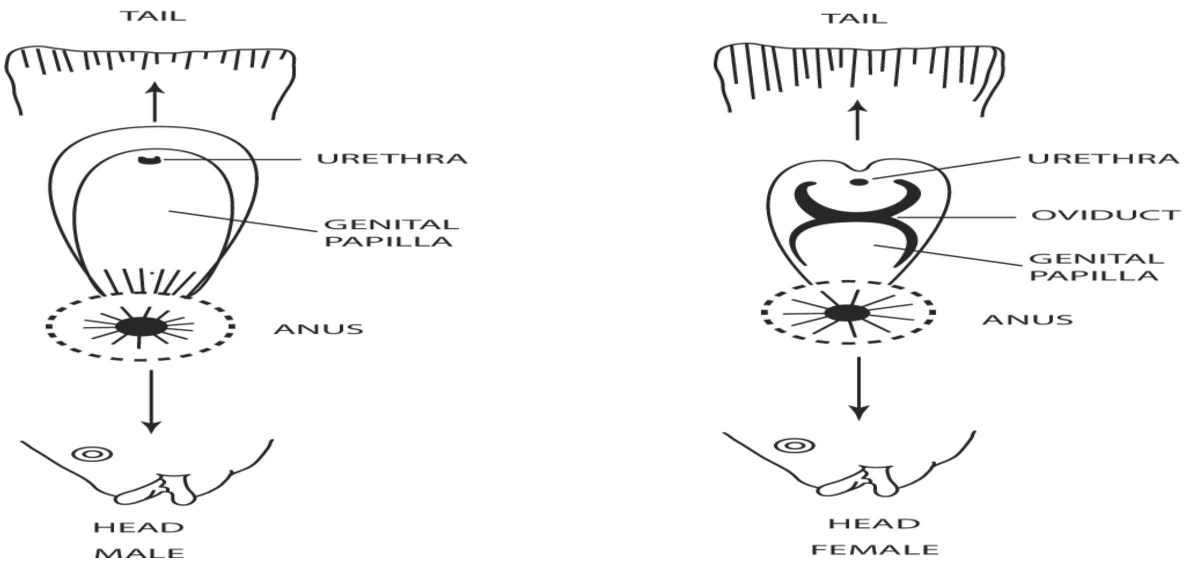

Figure 4. Male and female sex organs of Tilapia by (Nandial, 2004)

1.4.2.4 Skeletal system

The skeletal system consists of a bony structure with a complete series of vertebrae, ribs, and fin elements, providing both strength and flexibility (Trewavas, 1983).

Complementing this, the muscular system features lateral line muscles, known as myomeres, enable strong swimming abilities and quick responses, enhancing the fish's mobility (Stickney, 2006).

1.5 Physiological adaptations of Red Tilapia

Oreochromis spp exhibits multiple physiological adaptations that let it successfully survive diverse environmental niches thus providing valuable stability for aquaculture production methods (Angadi, 2024). The species shows four main physiological adaptations including osmoregulation together with temperature tolerance alongside oxygen utilization and stress response capabilities(Emad, 2022).

1.5.1 Temperature tolerance:

Red Tilapia exhibit several physiological adaptations to cope with temperature fluctuations (Refaey et *al.,* 2022). It can adjust their metabolic rate, reducing it at low temperatures to conserve energy and increasing it at higher temperatures to support growth and activity (Hamed et *al*., 2024). Under heat stress, it up –regulate HSP70 and other heat – shock proteins, which stabilize cellular proteins and prevent denaturation (Basu et al.,2002; Zhang et al., 2014). Behaviorally, it adjusts daily rhythms in temperature preference, favoring warmer environments during daylight and cooler waters at night, to maintain optimal physiological functioning (Vera et *al*., 2023).

1.5.2 Oxygen utilization:

Red Tilapia possess several adaptations that enable them to efficiently utilize oxygen, even in environments with low dissolved oxygen levels. Their gills have a large surface area, which enhances oxygen uptake and allows survival in waters with oxygen concentrations as low as 3 mg/L (Boyd, 2020). In hypoxic conditions, they can shift to anaerobic metabolism, relying on glycolysis for energy production and tolerating the buildup of lactic acid (Richards et al., 2009). Additionally, while not classified as true air-breathers, they can gulp air from the water surface to supplement oxygen intake when necessary (Graham, 1997).

1.5.3 Stress response

To cope with various environmental challenges like poor water quality, handling, and collection, a well-developed stress response system is activated. The release of cortisol, the primary stress hormone, helps mobilize energy reserves and suppress non-essential physiological functions during stressful events(Goes et *al*., 2019). Although stress can temporarily weaken the immune system, adaptive mechanisms allow for rapid recovery and resistance to infections (Tort, 2011). Additionally, antioxidant enzymes such as superoxide dismutase (SOD) and catalase are produced to neutralize reactive oxygen species (ROS) generated under stress conditions, helping to protect cellular integrity (Martínez-Álvarez et *al.*, 2005; Constantino, 2019).

1.5.4 Nutrition and digestion

As an omnivorous species, this fish has a highly adaptable digestive system that supports diverse feeding habits. It produces a broad spectrum of digestive enzymes, such as proteases, lipases, and amylases. That enable the breakdown of plant material, detritus, and animal proteins(García-Meilán et al., 2023). In addition, its gut microbiota plays a vital role in nutrient absorption and immune regulation, adjusting dynamically to changes in diet and environmental conditions (Ferguson et al., 2010).

1.5.5 Reproductive biology of Red Tilapia

(Oreochromis spp), reproduce polygamously, with males(10-30cm) creating and defending territories to attract females (EL-Sayed, 2006).
Spawming occurs when water temperature exceeds 20°C, influenced by latitude and season (Khamis et al., 2023). Females lay eggs in circular nests, fertilized externally by males (Lingamet *a*l, 2021), then incubate them in their mouths for 5-7 days until the fry can swim independently (Tave, 2000). Females can carry up to 1500 eggs (Khamis et *al*, 2023).

1.6. Tilapia growth:

A recent study has identified 32 developmental stages for Red Tilapia, covering embryonic, larval and early juvenile phases. The fish's bone structure keeps developing for 30 days after fertilization « dpf »

- ❖ Embryo stage (stages 1-18) includes seven steps, from a single cell to hatching.
- ❖ Larva stage (stages 19-25) has early and late growth phases.
- ❖ Juvenile stage (stages26-32) lasts until 30days old.

1.7. Diet and feeding habits:

Red Tilapia typically herbivorous or omnivorous, placing them mow on the aquatic food chain (El-Sayed, 2006). In its naturel habitat, its diet mainly consists of phytoplankton, which it filters through its gills. in aquaculture. tilapia is often fed formulated feeds designed to meet its specific nutritional needs (Cambell, 1978) .

It is also important to note that tilapia digestion is influenced by the oxygen concentration in the water. For example, studies have shown that tilapia stops digesting its food when the dissolved oxygen concentration drops below 3 mg/L (Abdel-Tawwab et al., 2015,Tran-Ngoc et al., 2016).

1.8. Nutritional requirement:

Fish, like all animals, require a well-balanced diet that includes all essential nutrients necessary for optimal growth. These nutrients needs var depending on the species ,sex, age and environment, larval development stages ,and overall health status of the individual (Vishnu, 2023).

Tilapia 's low trophic level and omnivorous diet make them relatively inexpensive to feed, unlike other finfish such as salmon, wich require high-protein- and high-fat diets based on costly protein sources like fish meal.(kamel, 2010).

1.8.1.protein

Juvenile Red Tilapia typically require a diet containing 25-35 % crude protein for optimal growth (Mohamed, 2020).

1.8.2.Lipids

Lipid levels are generally recommended to range between 5-15 %.They serve as an important energy source and contribute to the structure and function of cell membranes(Mohamed, 2020).

1.8.3.Carbohydrates

Carbohydrates are vital in fish nutrition,acting as a primary and cost-effective energy source.Although depend more on proteins than carbohydrates ,these nutrients are still essential for sustaining the energy required for dailly activities and growth.(Vishnu, 2023).

Table 1. Essentialamino acid requirement by(Shiau.2002,Fitzsimmous.(2005),El-Sayed (20006),Lim.2006)

Essential Amino Acid	Quantitative requirement %
Arginine	4,2
Histidine	1,72
Isoleucine	3,1
Leucine	3,3
Lysine	5,1
L-methionine	2,6
Threonine	3,7
Tryptophane	1,00
Valine	2,8

1.8.4.Fiber limitations

Dietary fiber should not exceed 10 % , as a higher levels reduce nutrirnt absorption and digestion efficiency

Plant based feeds often require fiber content adjustments to avoid compromising growth (LIM & Webster, 2006).

1.8.5.Vitamin supplementation :

Vitamins play a vital role in the growth,health,and cellular fuctions of Red Tilapia*(Oreochromis spp*) **(**Griesh, 2024**).**Vitamin E supports reproductive health by protecting cell membrane from oxidative damage,enhancing egg quality and larval development (Rohani, M. F.,2023).Vitamin C is essential for collagen formation,immune defense,and DNA protection through its antioxidant properties,helping the fish resist cellular stress (Noriega-Salazar 2014). B-complexe vitamins such as B6 is crucial for amino metabolism and energy production ;their levels must be ajusted depending on dietary protein and carbohydrate content(Nurfadilah, 2021). Vitamin D aids in calcium and phosphorus regulation,important for bone formation and overall growth (Hussein, 2021).

1.9.The common feed additives for Red Tilapia

1.9.1Enzymes (phytase,Amylase)

Phytase and amylase are enzymes added to fish diets to improve nutrient absorption. Phytase helps release phosphorus from plant ingredients (Javierre, J., et al. 2024) ,while amylase breaks down fiber ,making nutrients more accessible. Together,they boost growthand feed efficiency in tilapia(Maas, R., et al. 2020).

1.9.2.Probiotics

Probiotics supplementation in Red Tilapia diets enhance disease resistance in fish without necessarily improving growth performance (Wing-Keong Ng,2014). For example ,when exposed the red tilapia (*Oreochromis sp.*) to *Streptococcus agalactiae* ,with *Bacillus subtilis* being the most effective in providing protection. Ng et al.(2014).

1.9.3.Mineral supplementation

Mineral supplemntation plays a crucial role in optimizing growth and feed utilization in redTilapia farming .(Dato-Cajegas &Yakupitiyage,1996).Tilapia farming .(Dato-Cajegas &Yakupitiyage,1996).

Table 2. Essential minerals supplimentation and requirement for optimal growth in Red tilapia

Mineral	Importance	Recommended level
Phosphorus (P)	Growth and metabolism	0.35 %- 0.9 % of diet
Calcium (CA)	Bone formation	1 %
Maghnesium (Mg)	Enzyme activation	Not specified
Sodium (Na)	Osmoregulation	Not specified
Potassium (K)	Nerve and muscle function	Not specified
Iron(Fe),Zinc(Zn)	Physiological function	Trace amounts

1.9.4.Essential feed ingredients for optimal growth

The main feed ingredients for Red Tilapia typically include fish meal , oilseed meals (such as sunflower meal),rice meal ,corn,maize and wheat(Song et al., 2004). Wich togother provide a balaced diet rich in protein and carbohydrates essential for growth.(K.jauncey.2020).

1.9.5.Oilseed meals in the diet of Oreochromis spp

Oilseed meals are solid residues obtained after extracting oil from oilseed and are commonly used as plant-based protein sources in red tilapia feed.(Badran,2024).

- **Soybean meal** : one of the best plant protein sources,it has high digestibility and a good balance of essential amino acid.(Koffi, D.2023).
- **Peanut meal** : Rich in protein ,it is a good option for supplementing tilapia diets, but it must be properly processed to avoid aflatoxin contamination,wich can be toxic to fish (Sarr,2019).
- **Cottonseed meal** : It can be used in limited amounts due to the presence of gossypol,a substance that can be toxic at high levels (Yi-Rong.2008).
- **Rapeseed meal** : Contains good-quality proteins but should be used cautiosly because of antinutritional factors such as glucosinolates (Sallem 2021).

1.10 Major pathologies affecting Red Tilapia (*Oreochromis spp*)

Red Tilapia often suffer from bacterial and viral diseases that affect their growth and survival(Islam.S.I.2024).
The most common bacteria are *streptococcus agalactiae*,which causes high death rates(Zhang, 2021),and *pseudomonas aeruginose* known for skin and organ infections(Noor, 2021).A serios virus called tilapia Lake virus (TiLV) also affects Red Tilapia,weaking their immune system and causing high losse(Aich, 2022).Parasites ,such as monogeneans can also cause problmsin tilapia.(Isabel et *al*.2020).

2.Red Tilapia cultivation in algeria

2.1 Current status of aquaculture in algeria

Red Tilapia is gaining importance in algeria’s aquaculture sector, valued for its nutritious flesh and high market demand.known as the « aquaculture chiken »,it effienciently converts low-quality protein into high-quality meat,making it a profitable choice(Aps.DZ).
Oran wilaya : in el Karma, a private farm produces 300 tons annualy, with strong government support ,according to Maghni Sandid Menouar.
Khenchela wilaya ,a statue was installed as a symbol of the region’s commitment to aquaculture,promoting sustainablr fish production.

2.2 Farming Practice

The practice of framing in red tilapia farming refers to the methods and techniques used to efficiently grow Red Tilapia in different aquatic environments (Muir et al., 2001) in particular:

- Hatching :Managing breeding,collecting eggs ,and raising fry until yhey become fingerling(Mair et al., 1997 ; Liti et *al*., 2005).Fingerlings are sorted by growth before moving to grow-out tanks (Muir et al., 2001).
- Grow-out : raising fingerlings to market size while maintaining water quality and fish health (Muir et al., 2001).
- Harvesting :selecting,transferring,humanely killing,and processing fish into fillets (Pillay & Kutty, 2005)..Harvestingis seperate from hatchery equipment to ensure efficiecy (Diana, 2009; Tijani et al., 2018).

2.3 Economic importance of Red Tilapia

People call it the « aquatic chiken « because it can turn low-quality protein into high-quality protein.Plus, it is a good source of Omega-3 and vitamin D (Agence Presse Service, 2023).

Materials & methods

Materials and methods

The aim of this study is to evaluate the effects of different feed formulations on the growth performance, health status, and flesh quality of Red Tilapia within a circular aquaculture system.

1. The biological material:

We used in this study juvenile Red Tilapia *(Oreaochromis spp*) ,were obtained from **Ain Temouchent.**

2. Experimental design and Breeding system installation

The laboratory experiment was conducted using Red Tilapia *(Oreochromis sp).*

Figure 5. Red Tilapia ,taken during the acclimatization phase.

The experiment was carried out the molecular biology laboratury of IBN- khaldoun university in Tiaret. The breeding system was installed using plastic tanks with a capacity of 300 liters and 74 *79 cm diameters each.

A total of 4 tanks were used ,allowing for 4 experimental groups ,before starting the feeding trial. The Red Tilapia were acclimated in the laboratory for 20 days under the same environmental conditions as the experiment. During this period ,the fish were fed a local commercial diet from ONAB , provided three times a day. This routine allowed the fish to adapt gradually to the new environment and reduce stress.

The tanks were cleaned regularly ,and water quality parameters such as temeparature,ph,and oxygen levels were promptly removed to maintain healthy conditions in all tanks.

3. The filtration system

In our experiment, we used a basic water filter made from a plastic crate.It had two layers: the first layer was cotton wool to catch dirt and small particles, and the second layer was ceramic pieces to help clean water even more.

Figure 6. A filtration system.

4. Water evacuation:

We use the pump when the water becomes dirty.The basin is emptied using a plastic tube once a week or whenever needed.

5. Preparation of feed:

The feed preparation period lasted several days and involved collecting all necessary ingredients,this period was important to make sure everythings was ready on time and in good condition. The feed contains 25-30% crude protein,10% crude fat ,40-55% carbohydrates,and 9-10% moisture,7-9% CMV.

The four experiment were formulated on a specific ingredients to evaluate its effects on the growth and health of Red Tilapia ,the treatments included based on :

Carob:

(Ceratonia siliqua L) , commonly known as the carob tree, is an evergreen species of the leguminosae family,characteristic of the mediterranean basin (Brassesco, M2021).Carob derived products are valuable sources of dietary fiber,protiens ,natural sugars, and several bioactive constituents,notably polyphenols (Khatib, 2010),which contribute to thier antioxidant,anti-inflammatory effects(Testa, 2023).

Sunflower seeds:

Sunflower seeds are the small, nutrient-rich seeds harvested from the head of the sunflower plant (Helianthus annuus) (Puraikalan, 2023).They are a valuable source of essential nutrients, including healthy fats,protein, fiber,vitamin E, vitamin B1 and B5,selenium,and magnesium(Yamunadevi, 2023)

Vine by-products

Vine by -products refer to the residual materials drived from the cultivation and processing of graps (*Vitis Vinifera*), primarily consisting of grape pomace, seeds, skins, and stems . (Mathilde, 2021). These by- products are abundant in bioactive compounds such as polyphenols, flavonoids, and antioxidants, which have garnered attention for their potentiel applicattions in various industries, including agriculture and aquaculture (Martina, 2024).

Moringa:

Moringa oliefra (*Moringaceae)* is a highly valued plant known for its wide range of medicinal applications applications and exceptional nutritional content (Posmontier, B. 2011). Various parts of the plant contain essential minerals and serve as an excellent source of protein ,vitamins, beta-carotene,amino acids ,and diverse phenolic compounds(Alhakmani, F2013).

Spirulina:

Spirulina (*Limnospira platensis*) is a small,spiral-shaped microalga that grows in water (Maria, 2024) .It is well known for being very rich in nutrients like proteins (Boukhari N 2018),vitamins,minerals and antioxidants(Ramírez-Rodrigues2021).These natural compounds help support the body in many ways,such as boosting the immune system ,fighting inflammation,and improving energy(Sharon, 2024).

6. Steps of feed preparation :

1. Weighting all ingredients with precision

Figure 7. Weighting of feed ingredients.

2. Ground coarse products into fine powder

Figure 8. Ground ingredients for feed preparartion.

3. Mixed and homogenized with distilled water

4. The feed was steamed using a double boiler like the one in the picture, to help disinfect it before forming it into pellets.

5. Dried for 48 hours and packed with no air .

8. Monitoring fish growth during the experiment:

To study the growth performanc, we measured the fishe's weightusing a digital scale and size (length,width)with a custom-made ruler on every week.Thisregular monitoring help us track their development over time and compare the effects of different feed formulas on their growth.All measurements were taken carefully to ensure accurate andconsistent results.

9. Growth and feed use indicators:

To support our findings, we will also perform some statistical analyses, which will help us better undrestand and compare the results of each diet throughout the experiment.

10. Blood sampling for health analysis:

Blood samples were collected carefully from the fish to study their immune and biochemical responses .we used a sterile syringe to draw blood from the vien near the tail(called the caudal vein).The blood was put in special tubes,and each tube was labeled with the fish's group ,and stored properly until analysis.The procedure was done gently to avoid stressing the fish.

11.Tissue sampling

At the end of the experiment, we took some fish from each group to study their tissues under the microscope, First we anesthetized the fish using clove oil to keep them calm and avoid stress .Then ,we carefully removed the liver and part of the intestine using sterile tools.

The tissue samples were immediately fixed in 10% buffered formalin for a least 24 to 48 hours to preserve their cellular structure.

12. Lipid peroxidation :

The level of lipid peroxidation in the muscle tissue of Red Tilapia was evaluated using the TBARS (Thiobarbituric Acid Reactive substances) assay (Ohkawa et *al.,* 1979) , we analyzed 4 different samples,each with 3 repetitions,for a total 12 samples. For each sample , 1 gram of muscle tissue was homogenized with 10 ml of trichloroacetic acid (7.5% TCA).

After centrifugation at 3000 rpm for 10 minutes,the supernatant was mixed with an equal volum of thiobarbituric acid solution (0.67% TBA). The mixture was heated at 95 C° for 15 minutes , then cooled to room temperature. Absorbance was measured at 532 nm using a spectrophotometer. Then malondialdehyde (MDA) concentration was calculated and expressed in nmol per gram of tissue . The assay allowed for the comparison of oxydative stability between the different dietary treatments based on the degree of lipid peroxidation in the fish muscle.

Convert Absorbance to MDA (nmol/g tissue) :

We'll use this standard formula: MDA= A ×4× 10°6 / 1.56 × 10°5 = A × 25.64

13.Lipid extraction using the Folch method

Total lipids in tilapia muscle were extraxted using the Folch method (Folch et all 1957),a standard protocol based on solvent extraction ,Approximately 1 gram of fish muscle was homogenized with a mixture of chloroform and methanol (2 :1 v : v) .The homogenate was then filtred and mixed with a small volume of disttilled saline solution to allow phase seperation.

After standing,the mixture seperated into two layers : the lower chloroform phase containing the lipids, was carefully collected, and the solvent was evaporated .The remaining lipid residue was weighed to determine the total lipid content,expressed in miligrams per gram of tissue(mg/g) and after that per % of lipids .This method allows accurate and reproducible measurement of total fat in biological samples .

Results & discussions

1.Growth performance parameters :

Table (7) shows the indices values of growth performance and feed use. Results indicated significant variations among all dietary tested groups fed the experimental diets for five weeks.

Table 3 : Growth performance

Growth parameters	D1	D2	D3	D4
WG	192	97	166	191
DWG	5.65	2.85	4.88	5.62
SGR	1.36	0.82	1.28	1.44
FCR	2.66	4.99	3.37	2.57
FE	0.38	0.20	0.29	0.39

WG: Weight gain (g), DWG: Daily weight gain (g),SGR: Specific growth rate (%/day),FCR: Feed conversion ratio, FE: Feed efficiency .D:Diet.

The difference in weight gain and daily weight gain are notably remarkable between the four diets. The WG and DWG weresignificantly higher in D1compared to D4 (ONAB) which is the control diet. while thefeed conversion ratiowas most in D2 and D3 than D1.The specific growth rate was higher in D4 than D1 .However the feed efficiency was very lower in D2.Frequent observation was performed to find out where the maximum growth was taken place in the nine tanks.

1.1. weight gain:

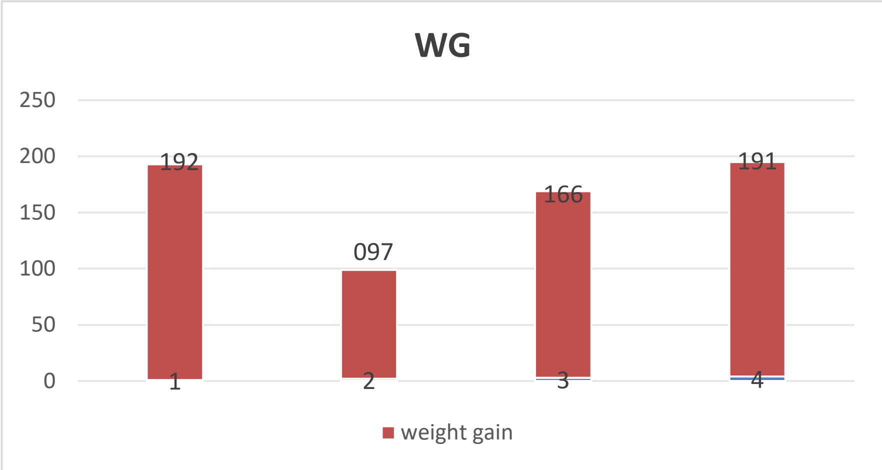

Figure 9. weight gain.

weight gain (g/fish) = (WF–WI), where WF = Final fish weight (gm) and WI = Initial fish weight (gm)

The histogram above illustrate the WG of red Tilapia fed the four experimental diets.The weight gain results showed clear differences among the four dietary treatments .The highest average weight gain was recorded in the group fedthe carob-supplemented diet reaching (192g),indicating its strong potential in promoting growth in Red Tilapia through improved digestion and antioxidant activity via polyphenols and essential fatty acids(Yilmaz, 2020).

This was followed by the group receiving thecommercial ONAB feed group (191g),which also demostrated a notable increase in body massserving as reliable referenceamong the other treatments, compared to the Spirulina group. The Spirulina group that ranked third in terms of weight gain, performed relatively well,with weight gains of (166g).While the pomace graps group recorded (97g),ranked among the lowest may be due to tannins and high fiber content from grape pomace and almond shells , which inhibit digestion and nutrient absorption which improved in similar studyfound that untreated grape pomace depressed body weight gain due to these anti-nutritional factors (Kumanda et *al*., 2019).

These results indicate that carob based diet had the most effective dietary additives for promoting weight gain in red Tilapia ,out performing both the control and other plant-based supplements.

1.2. Daily weight gain:

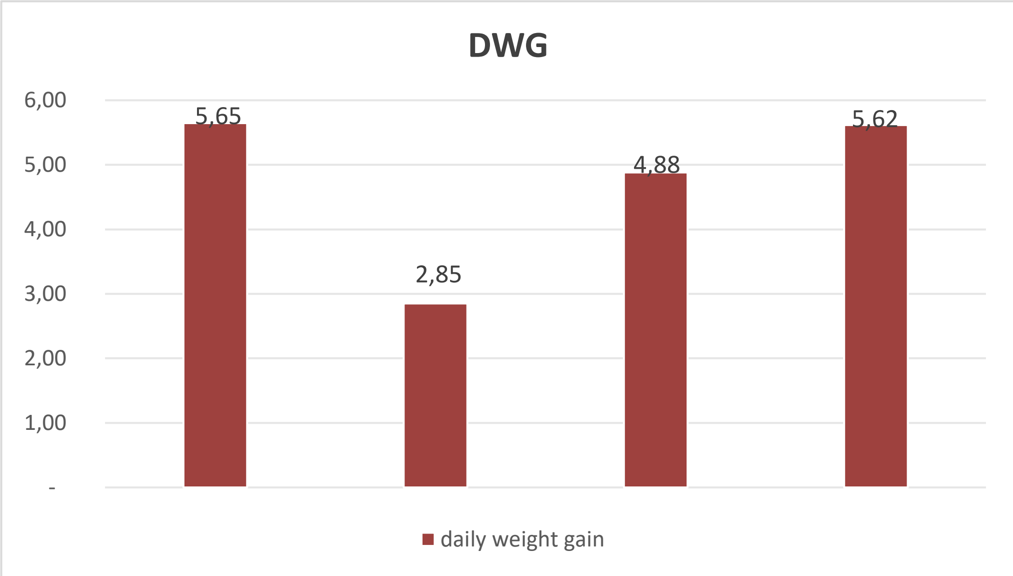

Figure 10. Daily weight gain histograme.

The Daily Weight Gain (DWG) wich represents the average amount of weight a fish gains per day, serving as a direct measure of growth performance and the nutritional effectiveness of the feed.

The results show the best performance in diet 1 (5.65g/day) comparing with a control diet 4 (5.62g/day) .The high DWG in diet 1 is likely due to easily digestibl energy sources like maize and carob, whose simple sugars and polyphenols improve nutrient absorption and gut health (Ikram et *al*., 2023). Diet 3 (4.88 g/day) achieved good results , possibly due to spirulina , which supplies high quality protein and phycocyanin, enhancing cellular metabolism and antioxidant defense as improved by(El-Araby et *al*., 2021).

Meanwhile, diet 2(2.85 g/day) showed the weakest performance ,likely limited by tannins and lignin from grape pomace and almond shells ,which impair digestive efficiency and reduce feedintake (Li et *al*., 2024).

1.3. Specific growth weight

Figure 11. specific growth weight.

Specific growth rate (SGR,% per day) =100 (ln final fish weight) −(ln initial fish weight)/ experimental days

The histogram below ilustrate the specific growth rate (SGR)which is a key indicator in aquaculture that measures the daily percentage increase in fish weight, helping assess growth performance and the effectiveness of different feed formulations of Red Tilapia fed with the four experimental diets.

Diet 4 gain the highest SGR (1.44%), followed closly by diet 1 with carob and sunflower seeeds, enhances nutrient absorption through polyphenols and unsaturated fatty acids contributing to stable growth

Diet 2 (0.82%) showed the lowest SGR, likely due to tannin rich grape pomace and indigestible fibers, which inhibit digestive enzymes and reduce nutrient assimilation, ultimately slowing protein turnover and growth as reported by (Martínez-Antequera et *al.,* 2024).

1.4. Feed conversion ratio:

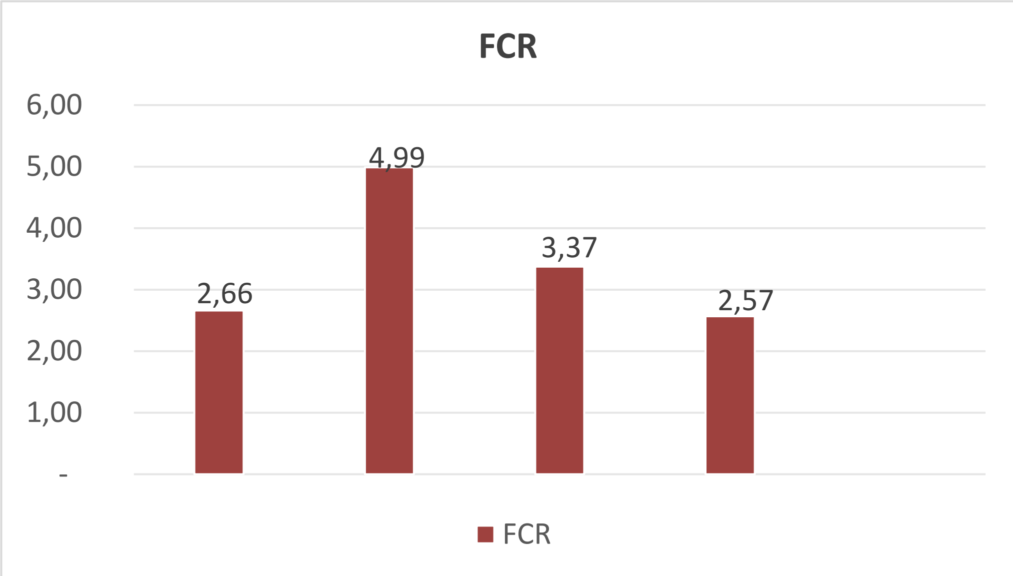

Figure 12. feed conversio ratio

Feed conversion ratio (FCR) =feed intake⁄weight gain

The FCR results show that the most efficient in diet 4(2.57) and diet 1(2.66), indicating better feed utilization.And the poorest FCR was in diet 2 (4.99) indicate a reduce feed digestibility and increase metabolic energy loss.

According to (Farrag and *al*, 2020) that feed efficiency parameters such as FCR, gave the best values in fish fed on the diet containing increasing dietary protein levels. A similar pattern of results was obtained in this study.

1.5. Survival rate:

(Number alive at the end of the period / Number alive at the start of the period) * 100

During the entire experimental period, the survival rate of Red Tilapia was 100% across all dietary treatments. No mortalities were recored in any of the nine groups, indicating that all feed formulation ,whether commercial ,plant-based , algae-based, or insect based were well tolerated by the fish .This high survival rate reflects the ovrall good health and adaptability of ed tilapia to the experimental conditions and confirms that none ofthe diets had any toxic or harmful effects.

1.6. Feed efficiency :

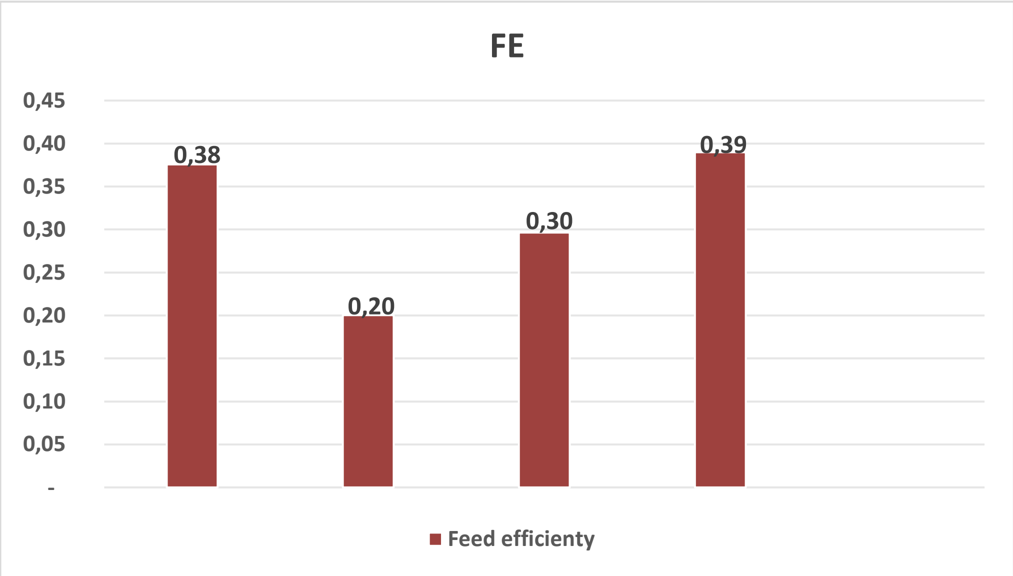

Figure 13. Feed efficiency.

The feed efficiency (FE) results match well with growth data carob gave the highest weight gain and SGR , also showed good feed use with FE values of 0.38 in line with their high growth.

In Diet 1 ,enriches with carob likely enhances FE(0.38) through the action of polyphenols as confirmed by (Hafsa et *al*., 2017).

The lowest FE was seen in diet 2 (0.20), likely due to high levels of tannins in grape pomace , which interfer with protein digestion and reduce the effective conversion of feed into growth , as documented by (Vinyard et *al*, 2021)in study showed that grape pomace proportion in cattle diets leading to impaired nutrients assimilation and reduce protein with slow growth.

1.7. Evolution of Red tilapia weight with different feeds during time :

The chart clearly shows a progressive increase in weight over time for all diets,with carob (D1)achieve the highest final weights by wheighing 6 .This consistent growth reflects the superior nutrient avaibility in these diets, rich in digestible carbohydrates, complete proteins, and metabolic cofactors, which support sustained anabolic processes.

The (D2) shows slow and flattened curve ,with minimal gains between weghings, indicating limited nutrient digestibility .

So the difference inweight gain and daily weight gain was found between the diets, might be due to the composition of each diet on its components and thier quality .

In fact the fish in D1 had fed on carob powder with 15% and sunflower seeds powder with 10%(as alternative source protein of soybean) whose rich onminerals(selenium,copper,…), vitamins (E, complex B,…) proteins .

Right behind the diet enriched with 5% of spirulina in D3 whichcontains high levels of proteins between 43% and 61%, depending on cultivation condition. As they contain all essential amino acids(Zhang et *al*., 2024) , which are essential for Red tilapia growth and often limiting in plant-based protein sources (Panahi, Y.and *a*l, 2016) also it is rich onvitamins(especially B-complexe) , minerals (iron,calcium) . The control group the fish in D4 (ONAB)had nourished on maiz that consists of low levels of protein 8–11% that is lack of essential amino acids tryptophan and lysine, and high levels of fibre.(Shikha,B.and *al*, 2019).

The high levels of fibre available at diet decreased the specific growth rate(O.B. Dioundick and D.I. Stom, 2003)andthis is what the results showed respectively on D2 : 0.15%

According to (Farrag and *al*, 2020) that feed efficiency parameters such as FCR, and gave the best values in fish fed on the diet containingincreasing dietary protein levels.A similar pattern of results was obtained in this study. Our results demonstrated that fish had fed on D2,,D3,and D4 recorded high levels of FCR respectively 4.98, 3.37, 3compared to the fish fed onD4 that recorded bad values of FCR (2.56).

According to (Winfree Robert A and Stickney Robert R, 2023) that FE attributable to changes in either protein or energy concentration. Significant interaction between protein and energy was also demonstrated in this study. The results showed that ,D4,D1 including cereals like : maiz, riz, and legumes like: sweet potato , which provide the energy due to the highest levels of starch and sugar (fructose,sucrose,glucose)can increase the FE values compared on D2.

1.8. Analysis of lipid content in fish flesh under different dietary treatments:

In this study, the lipid content in Red Tilapia flesh ranged from 1.33 to 2.24 , with the highest values recorded in fish fed diets enriched with spirulina(2.24%), which known for their rich content in essential fatty acids and amino acids, vitamins, and antioxidants(Vega et al., 2025), as a natural feed additives appear to positively influence lipid deposition or lipid metabolism in muscle tissue. Just closly folowed by the onab control group D4 with (2.08%°

Similarly,as for carob powder (*ceratonia siliqua*) , its inclusion in aquafeed may have contributed to improved nutrient utilization and energy storage Carob pods are rich in carbohydrates and polyphenols, which may enhance gut function and digestion,potentially leading to increased lipid retention in tissues (Othmen et *al*., 2020).

These results suggest that natural feed additives like microalgae and Carob can influence the lipid profile of red tilapia , either by increasing dietary lipid availability or enhancing metabolic efficiency.Compared to fish fed the standard diet, fish receiving these natural additives showed significantly higher lipid percentages ,and still within the normal physiological range for Red Tilapia (1-3%) (FAO/WHO,2016).

1.9. Final lipid oxydation (nmol/g)

Table 4 . TBARS Results.

Diet	Absorbance (A 532 nm)	MDA (nmol /g tissue)
1	0.046	1.17
2	0.054	1.38
3	0.037	0.94
4	0.082	2.10

We used the TBARS test to check how much lipid oxidation happened in the muscles of Red Tilapia fed with different diets. The absorbance values (at 532 nm) ranged from 0.037 to 0.082, which means the amount of MDA (a marker of oxidation) was between 0.94 and 2.10 nmol per gram of tissue. These values show that the oxidation level was low to moderate in all groups.

The group that received speruline showed low oxidation (like 0.94 nmol/g) wich has phycocyanin the major pigment protein complex ,highlighting its strong antioxidant effect(Castro-Gerónimo et *al.,* 2023) .Just behind followed by carobe known for their highest antioxidant activity (Cegledi et al., 2024).

In comparaison ,the fish that ate the ONAB control feed had the highest oxidation levels (up to 2.1) , showing that feeds without antioxidant plants are more affected by oxidation.

3. Biochemical and immune parametres:

Diet 2 with Grap pomace based showed eleveted hemoglobin 7.79 g/dL and hematocrit (27.57%), maybe due to iron and polyphenols in grape pomace(Iora et al., 2014.) .However , it had lower plasma proteins, and lysozyme, possibly due to tannins and lignin ,which reduce digestibility and immune enzyme expression as confirmed byPrusty et *a*l. (2007) .

Diet 3 showed relatively weaker performance compared to other diets, RCB count (1.37 $\times 10^6/\mu L$), hemoglobin (7.53 g/dL), and hematocrit (21.94%) were lower, suggesting limited support for blood oxygen transport.Additionally , plasma protein content. The low lysozyme suggest that high fiber and anti nutritional factors from bean may have partially impaired energy metabolism mentioned by Sharma, A. (2020).

Diet 4 the control group recorded a good results with highest hemoglobin (8.82 g/dL) and lipase (103.21 U/L), reflecting a well supporte doxygen transport thaks to its balanced formulation with maize, soybean .However , it showed moderate lysozyme (4.87 U/mL) and low plasma protein, indicating not enhanced immune stimulation and the high lipase level (103.21) indicate a stress in lipid digestion (Deng et *a*l., 2024),unlike diet 1.

In addition to that , the results in the present study detected that the seriously affected of different nutritional diets on biochemical parameters, which confirmed the potentially relationship between feeding systems and physiological responses in red Tilapia fish. In this respect, we found that carob based-diet increased significantly glucose with following ranges: (69.33-3.45 g/L),compared to ONAB diet which recoded (62.43-4.43g/L).This result was followed by grape-pomace whith(61.42- 6.98g/L) and spiriluna whith (57.85-6.70g/l) that demonstrate a remarkable increase in glucose. According to (Boonanuntanasarn and al,2018;Li and al,2021) the glucose levels increase with an elevated intake of dietary protein as spirulina which contains (60%-70%) of protein .

Previous studies have shown that high carbohydrate diet which based on high levels of starch as maiz on ONAB diet and carob based diet, as sweet potato in grape pomace diet can increase glucose blood levels.

Fish have the ability to convert excessive carbohydrates into lipids (Polakof and *al,*2012).

Digestive enzymes play vital role in breakdown nutrients and increasing their assimilation in the gastrointestinal tract (Hassaan et *al*. 2019). The current study reported that feeding fish with carob,grape pomace,spirulina and ONAB diets showed significant differences in Amylase activity were observed among different groups.

While Lipase activity recored high levels in ONAB Diet with(103,20- 19,05U/L)and carob-based diet groups with (119,48-7,73 U/L) and low lavels in the rest diet groups .

The fish have the ability to convert excessive carbohydrates into lipids(Polakof and al,2012). So this which found in this study.

CONCLUSION

Conclusion :

This study investigated the application of circular economy principles in aquaculture by evaluating the effects of alternative feed formulations on the zootechnical performance, biochemical responses, and nutritional quality of red tilapia (*Oreochromis sp.*). Four diets were tested: one based on commercial ONAB feed (control), and thre experimental diets formulated from local ingredients,Spirulina based feed (a microalga rich in protein and bioactive compounds) and carob based deit (*Ceratonia siliqua)*, known for its nutritional and antioxidant ppropertiesand the pomace gaps .

The results demonstrated that the carob-based feeds and spirulina produced the most significant improvements across multiple performance indicators. Fish fed with these formulations showed higher Specific Growth Rate (SGR), better Feed Conversion Ratio (FCR), and a 100% survival rate. Moreover, biochemical and hematological analyses indicated positive effects on blood parameters, lipid profiles, and tissue health, while the nutritional quality of the fish flesh,particularly protein and lipid content,was also enhanced.

These findings support the circular feed formulations using carob based feed can replace or supplement commercial feeds effectively. They not only reduce production costs and environmental pressure but also improve fish health and overall aquaculture sustainability.

Recommendations:

This study opens the door to a promising approach in sustainable aquaculture nutrition. Future research should go beyond growth performance to include comprehensive analyses of fish health, digestive efficiency, immune responses, and gut integrity. The use of functional ingredients such as carob, spirulina, and moringa deserves deeper investigation at the biochemical and molecular levels, particularly in relation to oxidative stress and nutrient assimilation. To maximize the real-world impact, it is essential to assess the economic viability, ensure ingredient standardization, and validate the results under practical farming conditions. Advancing this line of research could significantly contribute to affordable, eco-friendly aquaculture practices in resource-limited regions.

Perspectives:

This study opens promising avenues for future research, particularly in the fields of molecular biology and fish physiology. Further investigation is needed to understand how carob and spirulina based diet affect gene expression related to growth, metabolism, and immune response in fish. Additionally, studying the impact of these feeds on gut microbiota composition and histological structure of vital organs such as the liver and intestines could offer deeper insights into their mode of action. From an economic perspective, comprehensive cost-benefit analyses should be conducted to assess the profitability and scalability of producing these feeds locally. Moreover, their environmental impact on water quality and waste reduction should be evaluated. Finally, these alternative formulations could be integrated into sustainable aquaculture systems such as aquaponics or biofloc technology, reinforcing the principles of circular economy and resource efficiency in fish farming.

References

References :

Abd Elnabi, H. E., et al. (2024). The ability of the red tilapia (Oreochromis sp.) to tolerate different concentrations of unionized ammonia and water salinity levels. Egyptian Journal of Aquatic Agricultural and Food Sciences. Published on July 1, 2024.

Abdel-Tawwab, M., Hagras, A. E., Elbaghdady, H. a. M., & Monier, M. N. (2015). Effects of dissolved oxygen and fish size on Nile tilapia, Oreochromis niloticus (L.): growth performance, whole-body composition, and innate immunity. Aquaculture International, 23(5), 1261–1274.

Aich, N., Paul, A., Choudhury, T. G., & Saha, H. (2022). Tilapia Lake Virus (TiLV) disease: Current status of understanding. Aquaculture and Fisheries, 7(1), 7–17.

Alhakmani, F., Kumar, S., & Khan, S. A. (2013). Estimation of total phenolic content, in–vitro antioxidant and anti–inflammatory activity of flowers of Moringa oleifera

Amrul, N. F., Ahmad, I. K., Basri, N. E. A., Suja, F., Abdul Jalil, N. A., & Azman, N. (2022). A review of organic waste treatment using black soldier fly (Hermetia illucens). Sustainability, 14(8), 4565.

Angadi, P. (2024). The physiological adaptations during salinity stress in Tilapia fish: A review. Acta Entomology and Zoology, 5(2), 54-57.

Aquaculture, 26:1, 22-31.

Azaza, M.S., Saidi, S.A., Dhraief, M.N., & El-feki, A. (2020). Growth Performance, Nutrient Digestibility, Hematological Parameters, and Hepatic Oxidative Stress Response in Juvenile Nile Tilapia, Oreochromis niloticus, Fed Carbohydrates of Different Complexities. Animals, 10, 1913.

Azaza,M.S., et al.(2005)., et al.(2005)."Effet de la substitution de la farine de poisson par le tourteu de soja sur la croissance du tilapia de nil (*Oreochromis niloticus* L) élevé en milieu controlé ."*Reveu de Biologie Marine, 12(1),29-39.*

Badran, A.A., Magouz, F.I., Zaineldin, A.I. et al. Using a blend of oilseed meals in the diets of Nile tilapia (Oreochromis niloticus): effects on the growth performance, feed utilization, intestinal health, growth, and metabolic-related genes. BMC Vet Res 20, 529 (2024).

Basri, L., Nor, R. M., Salleh, A., Md. Yasin, I. S., Saad, M. Z., Abd. Rahaman, N. Y., … Amal, M. N. A. (2020). Co-Infections of Tilapia Lake Virus, Aeromonas hydrophila and Streptococcus agalactiae in Farmed Red Hybrid Tilapia. Animals, 10(11), 2141.

Boukhari, N., Doumandji, A., Ait Chaouche, F. S., & Ferradji, A. (2018). Effect of ultrasound treatment on protein content and functional properties of Spirulina powder grown in Algeria. Mediterranean Journal of Nutrition and Metabolism, 11(3), 243–254

Boyd, C. E. (2014). Nitrite toxicity affected by species susceptibility, environmental conditions. Global Aquaculture Advocate, 17(2), 64–65.

Boyd, C. E. (2020). Water Quality in Ponds for Aquaculture. Auburn University.

Boyd,C.E,McNEVIN,A.A,& David, R.P.(2022).The contribution of fisheries and aquaculture to the global protein supply.*Reviews in fisheries science & Aquaculture* ,30(1) 1613.

Brassesco, M. E., Brandão, T. R. S., Silva, C. L. M., & Pintado, M. (2021). Carob bean (Ceratonia siliqua L.): A new perspective for functional food. Trends in Food Science & Technology, 114, 310–322.

By Chhorn Lim, Ph.D. , Mediha Yildirim, M.S. and Phillip H. Klesius, Ph.D.(2022).

Cambell,D.(1978). "Formulation des aliments déstinés a l'elvage de Tilapia nilotica(L) en cages dans le lac de Kossou,Coté d'lvoire".

Ciezarek, A. G., Mehta, T. K., Man, A., Ford, A. G. P., Kavembe, G. D., Kasozi, N., Ngatunga, B. P., Shechonge, A. H., Tamatamah, R., Nyingi, D. W., Cnaani, A., Ndiwa, T. C., Di Palma, F., Turner, G. F., Genner, M. J., & Haerty, W. (2024). Ancient and recent hybridization in the Oreochromis cichlid fishes. Molecular Biology and Evolution, 41(7).

Constantino, R. V., Templonuevo, R. M. C., & Fajardo, L. J. (2019). Stress responses of red tilapia (Oreochromis spp.) to high ammonia levels. International Journal of Fisheries and Aquatic Studies, 7(6), 89-93. E-ISSN: 2347-5129, P-ISSN: 2394-0506.

Correia, D., Escarcega, B., Barreto, F., Mata, J., Del Rio, O., Viana, M., & Nishioka, A. (2019). Growth performance and body composition of hybrid red tilapia (Oreochromis mossambicus O. aureus) fed with different protein levels raised in saltwater. Latin American Journal of Aquatic Research, 47(5), 853–859.

Dan, C., Zhang, Y., & Wang, J. (2023). Dietary carotenoids enhance pigmentation and growth in red tilapia (Oreochromis spp.). Aquaculture Reports, 30, 101589.

Decano, E., Emplonuevo, R. M., & Cruz, E. V. (2020). Morphological and Physiological Changes in Red Tilapia (Oreochromis spp.) Subjected to High Temperature and Confinement Stress. Egyptian Academic Journal of Biological Sciences. B, Zoology, 12(2), 103–110.

El-Sayed, A. F. M. (2006). Tilapia Culture. CABI Publishing.

Fang, W., Huang, J., Li, S., & Lu, J. (2021). Identification of pigment genes (melanin, carotenoid and pteridine) associated with skin color variant in red tilapia using transcriptome analysis. Aquaculture, 547, 737429.

FAO(2020).Aquaculture feed and fertilizer resources .Fao fisheries technichal paper.

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Rome: Food and Agriculture Organization.

Ferrer-Gallego, R., & Silva, P. (2022). The wine industry by-products: Applications for food industry and health benefits. Antioxidants, 11(10), 2025.

Fitzsimmons, K. (2000). Tilapia: The Most Important Aquaculture Species of the 21st Century. World Aquaculture Society.

Griesh, A. M., El-Nahla, S. M., Aly, S. M., & Badran, M. F. (2024). Role of vitamin E supplementation on the reproductive and growth performance, hormonal profile and biochemical parameters of female hybrid red tilapia. Thalassas: An International Journal of Marine Sciences, 40, 1169–1178.

H.S. Abdo et al . Annals of Agric. Sci., Moshtohor, Vol. 57 (2) 2020

Hussein, S. Y., Metwally, M. A., & Asmaa, A. M. (2021). Effect of vitamin A and D_3 supplementation on the growth performance of Nile tilapia (Oreochromis niloticus). Assiut Veterinary Medical Journal, 67(173), 1–10. Received: March 13, 2021; Accepted: April 30, 2021.

Islam, S. I., Rodkhum, C., & Taweethavonsawat, P. (2024). An overview of parasitic co-infections in tilapia culture. Aquaculture International, 32, 899–927.

Javierre, J., et al. (2024). Effect of phytase supplementation in the diet of red tilapia (Oreochromis spp.) on production parameters.

K.jauncey.2000.Tilapias :Biology and exploitation,327-375.

Kadhim, M. J., & Hasan, M. H. (2021). A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of Thymus vulgaris Linn. Systematic Reviews in Pharmacy, 12(1), 36–43.

Khamis, M. A., et al. (2023). Reproductive Strategies of Tilapia Species in Aquaculture. Aquaculture Research, 54(1), 123-135.

Koffi, D. M., Kouadio, J. Y., Konan, K. J., & Aka, K. L. (2023). Effects of Substituting Soybean Meal by Unprocessed and Processed Peanut Cake Meal on the Growth, Body

Composition and Survival of Tilapia (Oreochromis niloticus). Journal of Biology and Life Science, 14(2), 103.

Kroupova, H., Machova, J., Piackova, V., Blahova, J., Dobsikova, R., Novotny, L., & Svobodova, Z. (2008). Effects of subchronic nitrite exposure on rainbow trout (Oncorhynchus mykiss). Ecotoxicology and Environmental Safety, 71(3), 813–820.

Liao, S. (2024, May 26). Spirulina – Benefits and Side Effects. WebMD

Lim, C.E. & Webster, C.D. (2006). Tilapia: Biology, Culture, and Nutrition.page506.

Lingam, S. S., et al. (2021). An Insight to Red Tilapia Breeding and Culture: A Farmer Advisory. Journal of Aquaculture Research & Development, 12(5), 1-8.

Liu,Y.,X.,Zhou,H.,Zhang,Y.,Huang,H. ,Cao,Y., Zhang,W.,Deng,J.,&Tan,B.(2022).Effect of different types of non-starch polysaccharides on groth,digestive enzyme activity ,itenstinal barrier fuction ,andantioxidant activity of tilapia .Aquaculture Reports,25,101198.

Lu, S., Taethaisong, N., Meethip, W., Surakhunthod, J., Sinpru, B., Sroichak, T., Archa, P., Thongpea, S., Paengkoum, S., Purba, R. A. P., & Paengkoum, P. (2022). Nutritional composition of black soldier fly larvae (Hermetia illucens L.) and its potential uses as alternative protein sources in animal diets: A review. Insects, 13(9), 831.

Maas, R., et al. (2020). Effect of enzymes (phytase and xylanase), probiotics (B. amyloliquefaciens), and their combination on growth performance and nutrient utilization in Nile tilapia.

Médal,F et Kaushik,S.(2009) "Les besoins nutritionenels des poissons en aquaculture"inra Productions Animales,22(3) ,203_210.

Mendes, A.R.; Spínola, M.P.;Lordelo, M.; Prates, J.A.M. ChemicalCompounds, Bioactivities, andApplications of Chlorella vulgaris inFood, Feed and Medicine. Appl. Sci.2024, 14, 10810

MOHD Zuabair Ashraf et al. (2024). Phytochemical and pharmacological study of Thymus vulgaris: A review. International Journal of Scientific Research in Science and Technology, july – august, 11(4) :190-201pages.

Montibus, M.; Vitrac, X.;Coma, V.; Loron, A.; Pinson-Gadais,L.; Ferrer, N.;Verdal-Bonnin, M.-N.;Gabaston, J.; Waffo-Téguo, P.;Richard-Forget, F.; et al. ScreeningofWood/Forest and Vine By-Productsas Sources of New Drugs forSustainable Strategies toControlFusarium graminearum and theProduction of Mycotoxins. Molecules2021, 26, 405.

Muchilisin,Z. A., Arisa,A.A., Muhammadar ,A.A., Fadli,N., Arisa,I.I.,&Siti(2016).Growth performance and feed utilization of keureling(Tor tambra) fingerlings fed a formulated diet with different doses of vitamn E (alpha-tocopherol).Fisheries& Aquatic Life,24(1),47-52.

Muin, H., Alias, Z., & Mohd Taufek, N. (2024). Co-culture of red hybrid tilapia and Gracilaria changii (Gracilariaceae: Rhodophyta): Effects on water quality, growth performance, and algal density. Phycologia, Published on April 12, 2024.

Ng, W.-K., Kim, Y.-C., Romano, N., Koh, C.-B., & Yang, S.-Y. (2014). Effects of dietary probiotics on the growth and feeding efficiency of red hybrid tilapia, Oreochromis sp., and subsequent resistance to Streptococcus agalactiae. Journal of Applied Aquaculture, 26(1), 22–31.

Nguyen, N. H., Hamzah, A., & Thoa, N. P. (2017). Effects of Genotype by Environment Interaction on Genetic Gain and Genetic Parameter Estimates in Red Tilapia (Oreochromis spp.). Frontiers in Genetics, 8.

Noor, N. M., Amal, M. N. A., Zamri-Saad, M., Yusoff, S. M., Siti-Zahrah, A., & Ina-Salwany, M. Y. (2021). Immuno-protective efficiency of feed-based whole-cell inactivated bivalent vaccine against Streptococcus and Aeromonas infections in red hybrid tilapia (Oreochromis niloticus × Oreochromis mossambicus). Fish & Shellfish Immunology, 113, 162–175.

Noriega-Salazar, A., Rivas, D., Malavé, A., Ramos, A., Maftei Aron, N., & Ramos-Villarroe, A. (2014). Influence of Vitamin C and E Enriched Feeding on Growth and Chemical Composition of Red Tilapia (Oreochromis spp.). The Annals of the University Dunarea de Jos of Galati, 38(1), 104–116.

NRC (2011). Nutrient Requirements of Fish and Shrimp. National Academies Press.

Nurfadilah, N., Jamaluddin, R., & Karim, M. Y. (2021). The Effect of Vitamin B Complex on Survival, Growth and Stress Resistance of Nile Tilapia (Oreochromis niloticus) Larvae. International Journal of Scientific and Research Publications, 11(11), 237–242.

Odongo, E.E., Bbosa, W.K.&Kahunde, P.K. (2024). Black Soldier Fly (BSF): A Sustainable Solution for Protein, Waste Management, and a Circular Bio-Economy. European Journal of Theoretical and Applied Sciences, 2(3), 822-834.

Popma, T. J., & Masser, M. P. (1999). Tilapia Life History and Biology. Southern Regional Aquaculture Center Publication No. 283.

Posmontier, B. (2011). The Medicinal Qualities of Moringa Oleifera. Holistic Nursing Practice, 25(2), 80–87.

Puraikalan, Y., & Scott, M. (2023, July). Sunflower seeds (Helianthus annuus) and health benefits: A review. Recent Progress in Nutrition, 3(3), 1–5

Rahman, A., Solichin, A., & Widyorini, N. (2021). Biological and environmental aspects of tilapia (Oreochromis niloticus) in the Jatibarang Reservoir, Central Java. IOP Conference Series: Earth and Environmental Science, 739(1), 012069.

Ramírez-Rodrigues, M. M., Estrada-Beristain, C., Metri-Ojeda, J., Pérez-Alva, A., & Baigts-Allende, D. K. (2021). Spirulina platensis protein as sustainable ingredient for nutritional food products development. Sustainability, 13(12), 6849

Rohani, M. F., Tarin, T., Hasan, J., Islam, S. M. M., & Shahjahan, M. (2023). Vitamin E supplementation in diet ameliorates growth of Nile tilapia by upgrading muscle health. Saudi Journal of Biological Sciences, 30(2), 103558.

Sallam, E. A., Matter, A. F., Mohammed, L. S., Azam, A. E., Shehab, A., & Mohamed Soliman, M. (2021). Replacing fish meal with rapeseed meal: potential impact on the growth performance, profitability measures, serum biomarkers, antioxidant status, intestinal morphometric analysis, and water quality of Oreochromis niloticus and Sarotherodon galilaeus fingerlings. Veterinary Research Communications.

Sallam, G. R., Helal, A. M., Mabrouk, H. A. H., Hermina, A. H. F. G., Habib, Y. J., Fayed, W. M., Dossou, S., El Basuini, M. F., & Shehata, A. I. (2024). Comparative impact of replacing fish meal with azolla on growth, water quality, and physiology of red tilapia fingerlings at varying salinities. Journal of Animal Physiology and Animal Nutrition, 108(5), 254.

Sarr,M .,Jean,F.,Amsatou,T.,&Rose,O.2019. Growth and survival of red Tilapia (Oreochromis aureus x Oreochromis mossambicus) fry fed on corn and soy meal, peanut meal and fishmeal enriched with spirulina (Arthrospira platensis). International Journal of Agricultural Policy and Research, 7(1), 1-9.

Siti Norita Mohamad, Wan Norhana Mohd. Noordin, Noor Faizah Ismail, Azhar Hamzah (2021). Red Hybrid Tilapia (Oreochromis spp.) Broodstock Development Programme in Malaysia: Status, Challenges and Prospects for Future Development. Asian Fisheries Science, 34: 73–81

Song, G.L.; Li, D.F.; Piao, X.S.; Chi, F.; Chen, Y. and Moughan, P.J. (2004). True amino acid availability in Chinese high-oil corn varieties determined in two types of chickens. Poult.,Sci., 83: 683-688.

Stickney, R. R. (2006). Aquaculture: An Introductory Text. CABI Publishing.

Tacon, A. G. J., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture, 285(1-4), 146–158.

Tave, D. (2000). Aquaculture and Breeding of Tilapia: Techniques and Practices. Aquaculture International, 8(1), 1-12.

Testa, M., Malandrino, O., Santini, C., & Supino, S. (2023). Nutraceutical and functional value of carob-based products: The LBG Sicilia Srl case study. In Case Studies on the Business of Nutraceuticals, Functional and Super Foods (pp. 107–120). Elsevier

Tilak, K. S., Veeraiah, K., & Raju, J. M. P. (2006). Effects of ammonia, nitrite and nitrate on hemoglobin content and oxygen consumption of freshwater fish, Cyprinus carpio (Linnaeus). Journal of Environmental Biology, 27(2), 333–336.

Waheed, M., Hussain, M. B., Saeed, F., Afzaal, M., Ahmed, A., Irfan, R., Akram, N., Ahmed, F., & Hailu, G. G. (2024). Phytochemical profiling and therapeutic potential of thyme (Thymus spp.): A medicinal herb. Food Science & Nutrition, 12(1),

Wing-Keong Ng, Young-Chul Kim, Nicholas Romano, Chik-Boon Koh & Si-YongYang (2014) Effects of Dietary Probiotics on the Growth and Feeding Efficiency of Red HybridTilapia, Oreochromis sp., and Subsequent Resistance to Streptococcus agalactiae, Journal of Applied

Yi-Rong Yue, Qi-Cun Zhou.2008. Effect of replacing soybean meal with cottonseed meal on growth,feed utilization, and hematological indexes for juvenile hybrid tilapia,Oreochromis niloticus×O. aureus. Laboratory of Aquatic Economic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, People's Republic of China.

Zhang, Z. (2021). Research advances on tilapia streptococcosis. Pathogens, 10(5), 558.

Zidan, E. M. (2022). Changes in Plasma Cortisol, Growth Performance and Other Stress Parameters in Nile Tilapia (Oreochromis niloticus) Subjected to Different Salinity Levels. Alexandria Journal of Veterinary Sciences, 73(1), 68-75.

Yilmaz, E. (2020). Effect of dietary carob (Ceratonia siliqua) syrup on blood parameters, gene expression responses and ammonia resistance in tilapia (Oreochromis niloticus). *Aquaculture Research*, *51*(5), 1903–1912.

Novodworski, J., Matos, É. J. A., Gonçalves, R. M., Bombardelli, R. A., & Meurer, F. (2025). Protein level reduction in corn and soybean meal-based diets supplemented with essential amino acids during the fattening phase of Nile tilapia. *Annals of Animal Science*.

Kumanda, C., Mlambo, V., & Mnisi, C. M. (2019). Valorization of red grape pomace waste using polyethylene glycol and fibrolytic enzymes: physiological and meat quality responses in broilers. *Animals*, *9*(10), 779.

Ikram, A., Khalid, W., Zafar, K. W., Ali, A., Afzal, M. F., Aziz, A., Rasool, I. F. U., Al-Farga, A., Aqlan, F., & Koraqi, H. (2023). Nutritional, biochemical, and clinical applications of carob: A review. *Food Science & Nutrition*, *11*(7), 3641–3654.

El-Araby, D. A., Amer, S. A., Attia, G. A., Osman, A., Fahmy, E. M., Altohamy, D. E., Alkafafy, M., Elakkad, H. A., & Tolba, S. A. (2021). Dietary Spirulina platensis phycocyanin improves growth, tissue histoarchitecture, and immune responses, with modulating immunoexpression of CD3 and CD20 in Nile tilapia, Oreochromis niloticus. *Aquaculture*, *546*, 737413.

Martínez-Antequera, F. P., Simó-Mirabet, P., De Las Heras, V., Román, M., Mancera, J. M., Martos-Sitcha, J. A., & Moyano, F. J. (2024). Grape pomace in diets for European sea bass: influence on oxidative status, intestinal microbiota, and fillet quality. *Aquaculture International*, *32*(6), 7771–7788.

Hafsa, S. H. A., Ibrahim, S. A., & Hassan, A. A. (2017). Carob pods (Ceratonia siliqua L.) improve growth performance, antioxidant status and caecal characteristics in growing rabbits. *Journal of Animal Physiology and Animal Nutrition*, *101*(6), 1307–1315.