UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO: Effects of dietary microalgae on growth performance, meat quality and gut morphometry in broiler chickens

Trabajo de integración curricular presentado como requisito para la obtención del título de Biólogo

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Urcuquí, octubre 2021

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Dedicatoria

Con mucho cariño, este trabajo se lo dedico principalmente a mi madre, quien ha sido mi guía a través de los años y el motor para alcanzar este logro.

A mi padre por su apoyo incondicional.

A mi familia quienes han sido mi fortaleza y soporte.

Francisco Gabriel Santacruz Hidalgo

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Francisco Gabriel Santacruz Hidalgo

Resumen

Este estudio buscó evaluar los efectos de diferentes especies de microalgas, administradas en dosis pequeñas (0.2%) sobre los parámetros de rendimiento del crecimiento, morfometría intestinal y calidad de la carne de aves destinadas a la producción. Un total de 72 pollos ROSS machos de un día de edad fueron separados en cuatro grupos experimentales. El primer grupo no tuvo la adición de polvo de microalgas y fue considerado como el control. El segundo, tercer y cuarto grupo fueron alimentados con polvo liofilizado de las microalgas *Tysochrysis lutea*, *Tetraselmis chuii* y *Porphyridium cruentum*, respectivamente, durante un periodo de 30 días. Los pollos alimentados con *Tysochrysis lutea* no presentaron efectos en los parámetros de crecimiento. Por otra parte, las aves alimentadas con *Tetraselmis chuii* y *Porphyridium cruentum* presentaron un efecto positivo en los parámetros de peso corporal, mejorando la ganancia media diaria en comparación con el grupo control. El índice de conversión alimenticia solo mejoro en las aves administradas con *Porphyridium cruentum*. Las secciones de duodeno e íleon correspondientes a los grupos alimentados con microalgas mostraron una mejoría en la arquitectura del intestino en relación al control. Los filetes de pollo correspondientes al grupo alimentado con *Tetraselmis chuii* obtuvieron una menor pérdida de peso por descongelación. En general, los pollos alimentados con *Tetraselmis chuii* y *Porphyridium cruentum* presentaron efectos positivos durante la crianza, y los resultados obtenidos sobre la morfometría del intestino se asociaron a una mejora en la ganancia de peso. Estos resultados muestran el potencial del uso de microalgas en la producción avícola.

Palabras clave:

microalgas; *Tysochrysis lutea*; *Tetraselmis chuii*; *Porphyridium cruentum*; pollos de engorde; rendimiento del crecimiento; morfometría del intestino; células caliciformes; perdida por descongelación.

Abstract

This study sought to evaluate the effects of different species of microalgae administered in small doses (0.2%) on growth performance, intestinal morphometry and meat quality of broiler chickens. A total of 72 one-day-old male ROSS broilers were separated into four experimental groups. The first group was left untreated and considered the control. The second, third and fourth groups were fed freeze-dried powder of the microalgae *Tysochrysis lutea*, *Tetraselmis chuii* and *Porphyridium cruentum*, respectively, for a period of 30 days. Birds fed *Tysochrysis lutea* showed no effect on growth parameters. On the other hand, chicks fed *Tetraselmis chuii* and *Porphyridium cruentum* showed a positive effect on body weight parameters, improving average daily gain compared to the control group. The feed conversion rate improved only in birds supplemented with *Porphyridium cruentum*. The duodenum and ileum sections corresponding to the groups fed with microalgae showed an improvement with regard to the architecture of the small intestine compared to the control group. Chicken fillets corresponding to the group fed with *Tetraselmis chuii* improved thawing loss. Overall, chickens fed with *Tetraselmis chuii* and *Porphyridium cruentum* exerted positive effects during rearing and the results obtained on intestinal morphometry were associated with body weight gain. These results show the potential of using microalgae in poultry production.

Key words:

microalgae; *Tysochrysis lutea*; *Tetraselmis chuii*; *Porphyridium cruentum*; broiler chickens; growth performance; intestinal morphometry; goblet cells; thawing loss.

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Introduction

Humans have used animal proteins as one of the main sources of nutrients (Larson & Fuller, 2014). During the last decades, and as a result of industrialization, the efficiency, healthiness and quality of foodstuffs derived from animals have improved (Karcher & Mench, 2017). Antibiotics, besides being used for preventing, controlling and treating diseases, have been also used as animal growth promoters (Kumar et al., 2019; Lin, 2014; Marshall & Levy, 2011). Their use for such purpose has been banned in the United States and some European countries (Hicks, 2020; Millet & Maertens, 2011). However, antibiotics are still misused worldwide, especially in rural regions in countries where no legislations have been specified for controlling antibiotic use (Cox et al., 2017).

There are several natural alternatives that are capable of improving growth and promoting health in animals such as chickens, which represent the main source of animal protein for humans (Alders et al., 2018). Various studies have shown that probiotics, plant extracts, and microalgae are useful as additives for improving growth performance and immune parameters in broiler chickens (Agostini et al., 2012; Amad et al., 2011; Amer et al., 2021; Fries-Craft et al., 2021; Liu et al., 2020, 2021; Omar et al., 2020; Šefcová et al., 2020; Xu et al., 2019; Zhao et al., 2020; Zhu et al., 2019). These studies have demonstrated that that treatments have exerted a positive influence on intestinal architecture, which ultimately improved growth.

Algae also represent an important source of nutrients. These phototrophic organisms produce a wide variety of nutritional compounds mainly exploited in aquaculture. However, their use in birds has been restricted principally to common species such as *Spirulina* or *Chlorella* (Coudert et al., 2020; Madeira et al., 2017). Nonetheless, no studies using important microalgae species such as *Tysochrysis lutea*, *Tetraselmis chuii* and *Porphyridium cruentum* have been published with regard to chickens.

1.1. Broiler Chickens

1.1.1. Growth parameters

Among the most common parameters that are measured to estimate the growth of broilers chickens we could find body weight gain, feed intake, and feed conversion ratio.

Weight gain is the result of nutrient intake ingested during broiler development. Some authors identify this factor as the most important to take into account during the breeding process (Bregendahl et al., 2002; Kabir et al., 2004; Odetallah et al., 2005). The cost of feeding represents approximately 70% of the total price of broiler rearing (Li et al., 2020), which directly influences the profitability of the product. For this reason, the parameters most commonly used by researchers to evaluate the efficiency of a feeding regimen are feed intake and feed conversion ratio.

Feed intake refers to the amount of feed consumed by a broiler during the rearing process. This parameter could be considered as the main external factor that determines the growth rate of an animal. Determinants that influence feed intake are particle size, feed shape, and grain type (Abdollahi et al., 2018). Normally, birds are fed *ad libitum*, however, there are studies that indicate that feed restriction may have an impact on bird growth (Lippens et al., 2000).

On the other hand, feed conversion ratio is an indicator that evaluates the amount of feed that has been transformed into body weight. This parameter is indirect, and is obtained through data of total feed intake and body weight gain, which must be measured during the poultry rearing process (Wen et al., 2018).

1.1.2. Intestinal morphometry

Morphometric studies concerning the growth of chickens are mostly based on the measurement of villus height and crypt depth of duodenal, jejunal, and ileal sections (Wilson et al., 2018). The small intestine is the area where most nutrients can be absorbed (Barbeito, 2014; Goodman, 2010). An increased surface implies an enhanced absorption of available nutrients, which ultimately can have an influence on body weight (Obajuluwa et al., 2020).

The epithelial zone of the chick small intestine represents a complex system where nutrient absorption takes place. Here we could find a variety of cells including enterocytes, stem cells, enteroendocrine cells, transit-amplifying cells, paneth cells, stem cells and goblet cells (Figure 1) (Zhang et al., 2019). The latter secrete mucins and other glycoproteins that constantly interact with bacteria trapped in the mucosa of the intestinal wall, which functions as a protective and transport barrier for immune cells (activated T cells, plasma cells, dendritic cells and macrophages) (Liu et al., 2020). Goblet cells also secrete antimicrobial peptides, chemokines and cytokines, thus having an influence in the innate immune reaction (Knoop & Newberry, 2018).

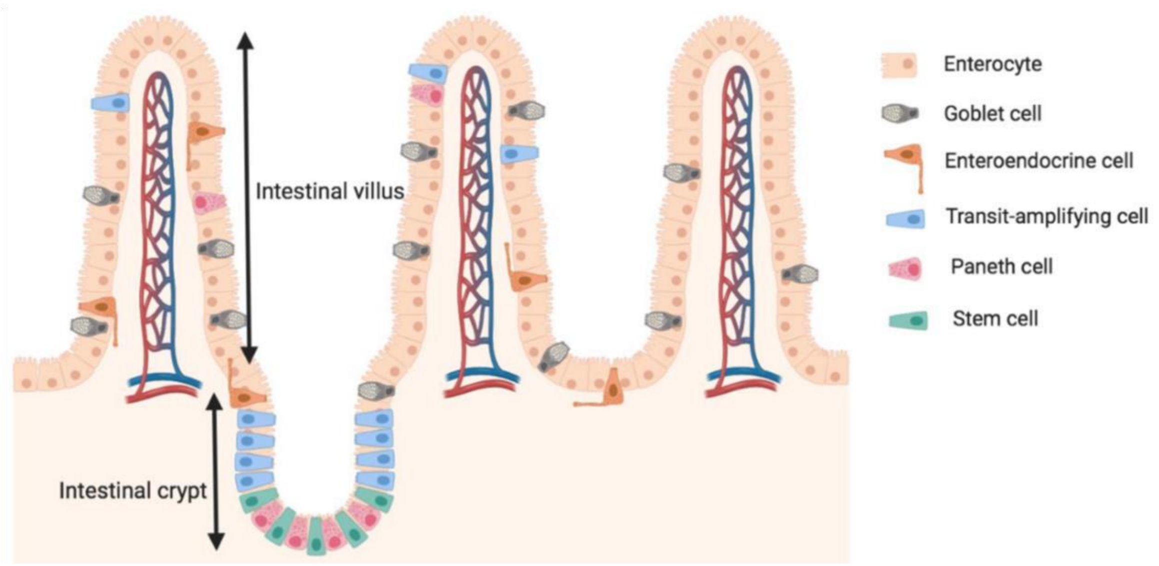

Figure 1: Chicken small intestine epithelium. Adapted from *Chicken small intestinal epithelium model* by Zhang et al. (2019)
The invaginations corresponding to the intestinal crypts are characterized as the zone of cell differentiation. Stem cells, transit-amplifying cells, and paneth cells are located here. On the other hand, the finger-like structures correspond to the intestinal villi where mature cell populations such as enterocytes, goblet cells and enteroendocrine cells are found.

1.1.3. Meat quality

Meat quality refers to the characteristics of the food derived from the animal, especially breast fillet quality (Miller, 2002). There are both qualitative and quantitative factors considered for evaluation. In the former, the most important include palatability, color, and odor (Fletcher, 1999; Miezeliene et al., 2011; Miller, 2002). In the latter, pH, bacterial count, and water holding capacity are commonly considered (Cramer et al., 2018; El-Bahr et al., 2020; Holdstock et al., 2014).

Water holding capacity is considered of particular importance. Muscle tissue loses water due to changes in the volume of muscle myofibrils and protein denaturation. When the muscle tissue is healthy, it retains more water, expelling less fluid into the fiber bundles and losing a smaller amount of weight due to fluid retention (Honikel, 1998). Water retention capacity is normally assessed by cooking loss, where the muscle is exposed to high temperatures inducing water loss by evaporation. Also, thawing loss is an important parameter, again properties of the muscle tissue influence water retention (Honikel & Hamm, 1994).

1.2. Microalgae as Feed Enhancers

Algae are photosynthetic, aquatic autotrophic organisms divided in macroalgae and microalgae. The former correspond to organized multicellular organisms, while the latter are generally unicellular and do not form complex structures (Figure 2) (Guiry, 2012). Microalgae are microscopic photosynthetic organisms that present a wide variety of shapes, sizes and colors (Levasseur et al., 2020). These microorganisms are characterized by their short generation times and their ability to multiply exponentially when subjected to favorable environmental conditions (Vale et al., 2020).

Microalgae produce fatty acids, carbohydrates, proteins, vitamins, lipids, minerals and other bioactive compounds such as pigments or antioxidants. These vary in quantity depending on the species, cultivation method and environmental conditions (Madeira et al., 2017). These compounds have been proved to be useful in animals as growth promoters and modulators of the immune response (Coudert et al., 2020). Generally, the use of microalgae has been destined for aquaculture. However, their use as a feed additives within poultry farms has increased in recent years (Abdelnour et al., 2019; Wafaa, 2020).

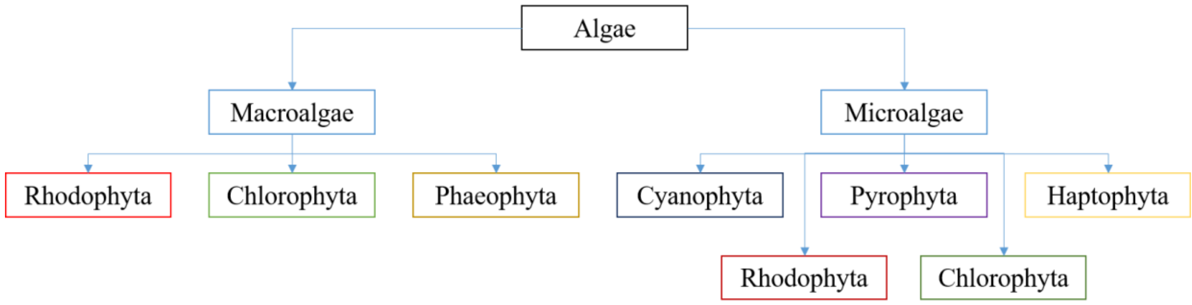

Figure 2: Algae classification. Inspired from (Javed et al., 2019; Levasseur et al., 2020).
Some representative phyla of algae such as Rhodophyta and Chlorophyta, have members in both divisions.

1.2.1. Fatty acids

Fatty acids are biological molecules of lipidic nature, formed by linear hydrocarbon chains. Studies on various types of microalgae have shown that there are about 135 different types of fatty acids present in these microorganisms (Maltsev & Maltseva, 2021).

Normally, the type of fatty acids most commonly used in the poultry industry are poly-unsaturated fatty acids. These types of fatty acids are of great importance in meat production, because they are capable of modifying the amount and place of deposition of accumulated fat in broilers (Crespo & Garcia, 2002); as such, they improve growth and meat quality parameters (Miller, 2002).

1.2.2. Dietary fibers

Dietary fibers are edible compounds present mainly in the cell walls of microalgae. Some examples are polysaccharides such as pectin or cellulose (Dvir et al., 2000); and oligosaccharides such as the acidic oligosaccharides III-O1, III-O2 (Geresh et al., 2009). These substances are hardly digestible by animals, but are partially fermented by bacterial colonies located in the small intestine. Therefore, these compounds play a special role in the development of gut microbiota, which has been shown to be beneficial (Patel et al., 2021). Fermentation processes generate short chain fatty acids (e.g. butyrate) that are known to exert positive effects on the epithelia of the small intestine (Koh et al., 2016). For instance, short chain fatty acids induce enterocyte division and goblet cell differentiation (Pearce et al., 2020; Piekarska et al., 2011).

1.2.3. Bioactive compounds

Other bioactive compounds found in microalgae include phenols, pigments and vitamins. The effects of polyphenol supplementation in poultry diets are not completely clear; however, it is known that these molecules interact directly with other relevant components such as proteins and polysaccharides. They have been associated with alterations of intestinal physiology, pH, intestinal fermentations, and bile secretions (Gopi et al., 2020; Matos et al., 2019). Various pigments are synthesized by microalgae, the most important are carotenoids (Matos et al., 2019). It has been shown that these compounds play a role as a precursor of vitamin A. They are used for improving pigmentation of the skin of chickens and yolks of eggs. In addition, they exert important effects in the avian immune response (Koutsos et al., 2003; Matos et al., 2019; Riley et al., 2021).

1.3. Microalgae Used in this Experimentation

The species *Tysochrysis lutea*, belonging to the golden-brown microalgae family, is part of a group of auspicious marine haptophytes, with an important production of poly-unsaturated fatty acids (Alkhamis & Qin, 2016; Rasdi & Qin, 2015). In addition, this species is rich in pigments such as fucoxanthin with nutraceutical and pharmaceutical applications (Gonçalves de Oliveira-Júnior et al., 2020). On the other hand, *Tetraselmis chuii*, a microalga belonging to the green algae family, is a species known for its simple cultivation and high nutritional value (Bonilla-Ahumada et al., 2018). This species accumulates high amounts of compounds such as poly-unsaturated fatty acids, proteins and pigments (Lu et al., 2017). Finally, among the family of red algae, we focus on the species *Porphyridium cruentum*, which is capable of producing high amounts of sulfated polysaccharides, lipids and poly-unsaturated fatty acids (Erol et al., 2020; Hu et al., 2018), known for its antioxidant, anti-inflammatory role and as a cytotoxic agent (Casas-Arrojo et al., 2021; Patil et al., 2007). These characteristics are promising for food industry.

2.Problem Statement

Microalgae are biological organisms with a high nutritional capacity. Despite this, several species widely used in aquaculture have not yet been tested in terrestrial animal husbandry. Within poultry, there are few alternatives to fatten broilers naturally. For this reason, *Tysochrysis lutea*, *Tetraselmis chuii* and *Porphyridium cruentum* are proposed as supplements to improve growth parameters, meat quality and gut morphometry in broilers.

2.1. General Objective

The general objective of this project is to evaluate the effects on growth performance, gut morphometry and meat quality in broilers fed with three microalgae species traditionally used in aquaculture: *Tysochrysis lutea*, *Tetraselmis chuii* and *Porphyridium cruentum*.

2.2. Specific Objectives

i. Asses statistically body weight gain, daily feed intake and feed conversion ratio of experimental groups of broilers fed with microalgae powder.
ii. Analyze the small intestine morphometry in broilers fed with microalgae and a control group, by measuring the length of the villi and the depth of the crypts captured from images of duodenum and ileum.
iii. Quantify the number of goblet cells present in images captured from the small intestine for the evaluation of the immune response of broilers.
iv. Evaluate the cooking loss and thawing loss corresponding to meat quality parameters.

3.Methodology

This experimental work was conducted at "Facultad de Medicina Veterinaria y Zootecnia de la Universidad Central del Ecuador". All experiments related to broiler rearing were conducted following the standards and guidelines for poultry management provided by the "Agencia de Regulación y Control Fito y Zoosanitario" (AGROCALIDAD, technical resolution No. 0017). In addition, the experimental protocol of this project (Reference Number: 2020-008) was reviewed and approved by the "Comité de Ética Sobre el Uso de Animales en Investigación y Docencia de la Universidad San Francisco de Quito".

180 male chicks (ROSS 308), which registered an average of 50 ± 5.8 grams, were randomly divided into four experimental groups. Each experimental group was divided into nine subgroups consisting of five broilers (Figure 3). The subgroups were considered the experimental unit, as they were housed separately and independently exposed to treatment conditions. For measurements, two birds at random were selected for each of the subgroups, for a total of 72 chicks (18 for each experimental group).

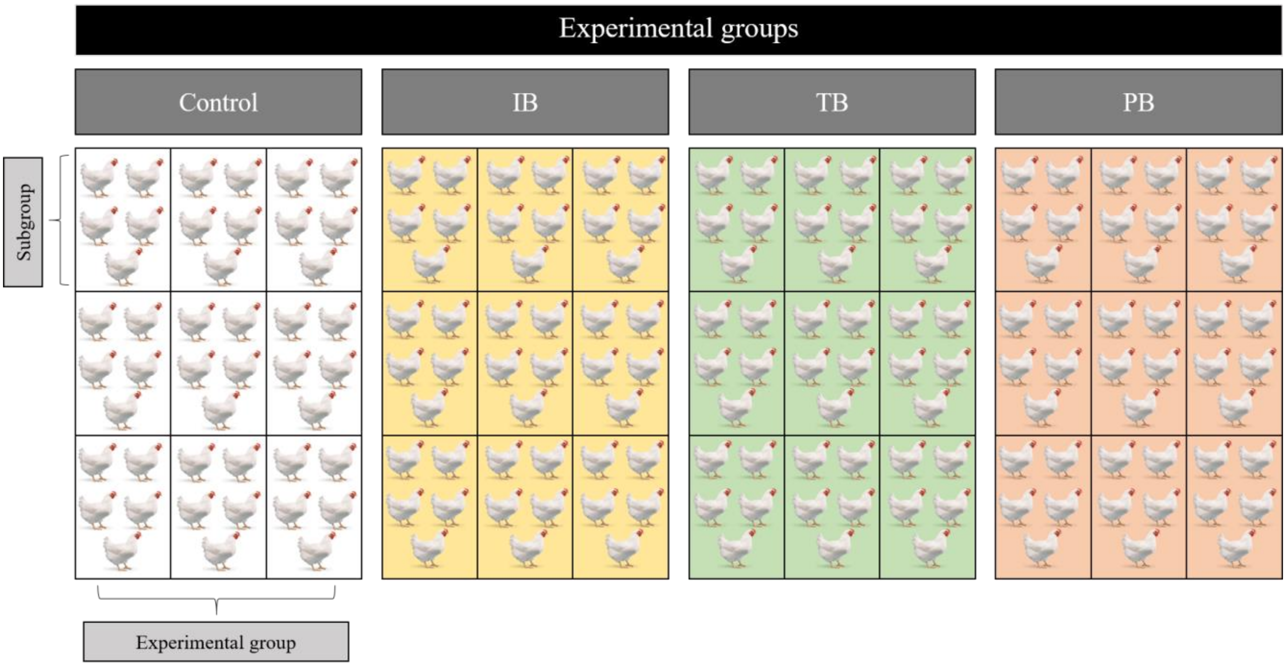

Figure 3: Illustrative representation of the experimental design. (not on scale). IB: Broilers fed with *Tysochrysis lutea*, TB: Broilers fed with *Tetraselmis chuii*, PB: Broilers fed with *Porphyridium cruentum*. Birds belonging to each experimental group were housed in separate pens of 3 m^2, and divided into nine subgroups of 1 m^2. Data collection for the different experiments was carried out by selecting two birds at random from each of the subgroups, giving a total of 18 chicks (sampling units) for each experimental group.

Chicks received basal diets with nutritional components for broilers in the starter (1 to 11 days) and finisher (12 to 30 days) stages, whose nutritional components are detailed in Table 1. The diets were free of probiotics, antibiotics or anticoccidiostats.

Table 1: Feed components of starter (1 to 11 days) and finisher (12 to 30 days) diets.

Feed components								
Components	**Control**		**IB**		**TB**		**PB**	
	1 to 11 days	12 to 30 days	1 to 11 days	12 to 30 days	1 to 11 days	12 to 30 days	1 to 11 days	12 to 30 days
Microalgae-derived protein (%)	0.00	0.00	0.08	0.08	0.07	0.07	0.07	0.07
Microalgae-derived fat (%)	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01
Microalgae-derived crude ash (%)	0.00	0.00	0.05	0.05	0.06	0.06	0.06	0.06
Microalgae-derived carbohydrate, fibre, rest of biomass (%)	0.00	0.00	0.06	0.06	0.06	0.06	0.06	0.06
Ground corn (%)	55.88	69.35	55.77	69.21	55.77	69.21	55.77	69.21
Soybean paste (%)	38.23	25.47	38.15	25.42	38.15	25.42	38.15	25.42
Palm Oil (%)	2.07	2.14	2.07	2.14	2.07	2.14	2.07	2.14
Calcium carbonate (%)	1.18	0.86	1.18	0.86	1.18	0.86	1.18	0.86
Monocalcium phosphate (%)	1.07	0.62	1.07	0.62	1.07	0.62	1.07	0.62
Industrial Salt (%)	0.41	0.19	0.41	0.19	0.41	0.19	0.41	0.19
L-Methionine (%)	0.34	0.3	0.34	0.30	0.34	0.30	0.34	0.30
Lysine Sulphate (%)	0.17	0.26	0.17	0.26	0.17	0.26	0.17	0.26
Sodium bicarbonate (%)	0.17	0.19	0.17	0.19	0.17	0.19	0.17	0.19
Vitamin and mineral premix	0.23	0.26	0.23	0.26	0.23	0.26	0.23	0.26
Antimycotoxin (%)	0.1	0.08	0.10	0.08	0.10	0.08	0.10	0.08
Mycotoxin Sequestrant (%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Choline chloride (%)	0.04	0.1	0.04	0.10	0.04	0.10	0.04	0.10
L-Threonine (%)	0.03	0.06	0.03	0.06	0.03	0.06	0.03	0.06
Antioxidant (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Phytase (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Copper sulphate (%)	-	0.04	-	0.04	-	0.04	-	0.04

Different diets were assigned to each of the experimental groups. The first experimental group was fed the basal diet with no addition of microalgae, and was therefore considered the control group. The broilers from the second, third and fourth groups were fed a diet enriched with lyophilized microalgae powder, which was introduced in a proportion of two grams per kilogram of feed. In the group two (IB), *Tysochrysis lutea* microalgae powder was added; in group three (TB), *Tetraselmis chuii* microalgae was added; and in group four (PB), *Porphyridium cruentum* microalgae was administrated. Broilers were able to eat and drink freely throughout the experimental stage. The experiment had a duration of 30 days.

The lyophilized powder of *Tysochrysis lutea*, *Tetraselmis chuii* and *Porphyridium cruentum* species was obtained from Necton S.A., Olhão, Portugal (https://necton.pt/). The biomass of *Tysochrysis lutea* contained 40% crude protein, 6% crude fat and 23% crude ash. This species is characterized by containing both chlorophylls a and c, as well as carotenoids such as fucoxanthin, responsible for its golden-brown coloration (Lovejoy, 2020). *Tetraselmis chuii* powder was composed of 35% crude protein, 5% crude fat and 30% crude ash. Organisms belonging to the genus *Tetraselmis* are characterized by the presence of chlorophylls a and c, and the carotenoids violaxanthin, antheraxanthin, zeaxanthin (Lewis & McCourt, 2004). Finally, the biomass of *Porphyridium cruentum* contained 35% crude protein, 5% crude fat and 31% crude ash. *Porphyridium* species contain chlorophyll a, α- and β-carotene, lutein and zeaxanthin. Also, these microorganisms are characterized by the production of water-soluble pigments such as phycobilins (Lima et al., 2021; Wehr et al., 2015).

Chickens were reared on the floor with a space allowance of one subgroup (five broilers) per square meter and a relative humidity maintained between 50-60%. Five experimental periods were determined during which the broiler rearing conditions were changed: time lapse one (TL1), from day 1 to day 6; time lapse two (TL2), from day 7 to day 13; time lapse three (TL3), from day 14 to day 20; time lapse four (TL4), from day 21 to day 27; and time lapse five (TL5), from day 28 until the end of the experiment (day 30). The environmental temperature was maintained around 30-32 °C during TL1 and was gradually reduced by 3 °C each week, so that the chicks were exposed to temperatures between 27-29 °C in TL2, between 24-26 °C in TL3, 21-23 °C in TL4 and 19-21 °C in TL5. Regarding the light regime, broilers were exposed to a regime of 23 hours of light (at 30-40 lux intensity) and 1 hour of darkness during the TL1, during TL2, TL3, TL4 and TL5 the light regime was changed to 21 hours of light (at 5-10 lux intensity) and 3 hours of darkness. Environmental and housing conditions were in accordance with the ROSS Broiler Management Guide (Aviagen, 2018).

3.1. Growth Performance Parameters

Weights were recorded by selecting two chicks at random for each of the subgroups, the value of weights was averaged and annotated for a total number of nine sampling units ($N = 9$) for each experimental group. The weighing process was carried out during the days of temperature change (first day of TL2, TL3, TL4 and TL5). In addition, weight of the twenty-ninth and thirtieth days were also registered. Feed consumption was recorded daily.

3.2. Morphometrical Analyses

One chick for each subgroup was selected on the day 30. The animals were euthanized by electrical stunning and bleeding, and samples of the small intestine were collected. Sections of duodenum and ileum of three centimeters each were fixed with a 10% formalin solution for 48 hours. Subsequently, the samples were dehydrated by serial washes with ethyl alcohol (70-100%), diaphanized with xylol and finally embedded in kerosene blocks. Then, the blocks were cut into three longitudinal sections of five-micrometer-thick slices using a microtome, stained with hematoxylin and eosin, and placed in slides for evaluation in the microscope.

Images were analyzed using Motic Images Plus 2.0 image processing software (Motic, Hong Kong, China). For the morphometric analysis, villus height and crypt depth were determined in uninjured segments of the duodenum and ileum. For each segment, a minimum of six villi in good condition with an intact lamina propria were selected.

Analysis of the relationship between villus height and crypt depth was calculated by dividing the mean villus length by the mean crypt depth in each of the samples as described by Nain et al. (2012). Moreover, the total number of goblet cells present per 100 epithelial cells in six intact villi was counted as described in previous research (Liu et al., 2020).

3.3. Meat Quality Parameters

Samples of approximately 10 grams were sectioned from the major pectorals of nine birds per experimental group at the end of the experiment. For the study corresponding to cooking loss, the fillets were placed in thermotolerant plastic bags which were immersed in a water bath until reaching 70° C, after which they were placed in ice to be cooled to 5° C and finally weighed. On the other hand, for the measurement of thawing loss, the breast samples obtained were dried, trimmed and stored at a temperature of -18 °C for one week. At the end of the established time, the fillets were thawed at a temperature of 5 °C for 1 day and then weighed. The differences between the initial and final weights were considered loss values. This experiment was carried out under the procedures previously described by El-Bahr et al. (2020).

3.4. Statistical Analyses

Statistical analyses for growth performance and gut morphometry characteristics were performed in MATLAB® (MathWorks, Natick, MA, USA) version 9.9.9341360 (R2016a). Normality and homogeneity of variance was tested using the Shapiro-Wilk test and Levene's test, respectively. Data with a normal distribution and homoscedasticity were subjected to a one-way analysis of variance to determine significant differences between experimental groups, together with a Tukey post hoc test. Data with a normal distribution and heteroscedasticity were analyzed using Welch's ANOVA and Welch's t-test. For non-normally distributed and homoscedastic data, the Kruskal-Wallis test and Dunn's test were used.

4.Results

4.1. Growth Performance

Regarding body weight, birds of the TB and PB groups weighed more ($p<0.05$) than those of the control on the day of slaughter. However, on previous days, other differences between the experimental groups were detected as shown in Figure 4.

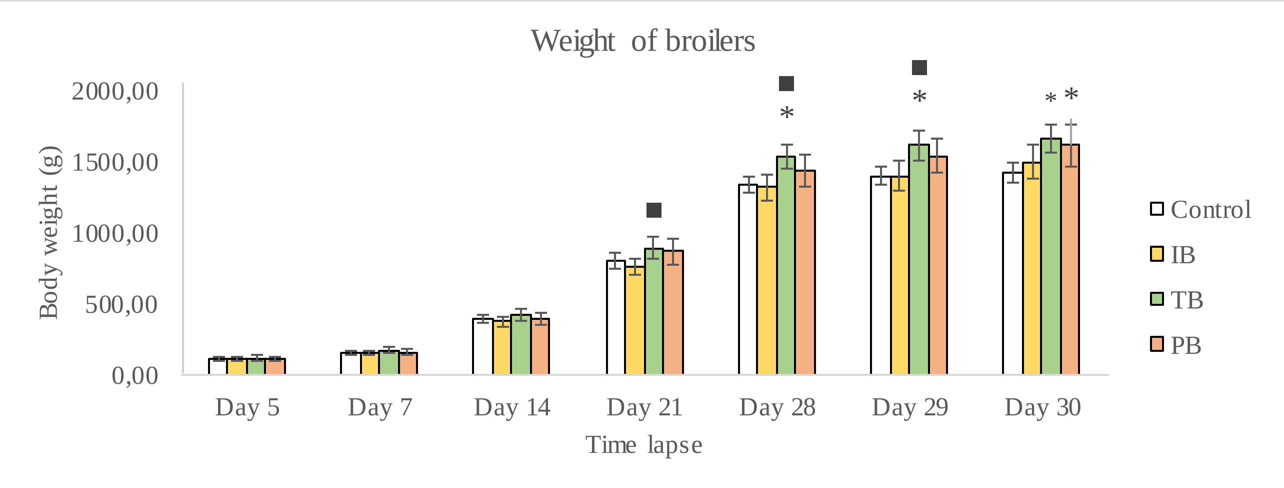

Figure 4: Body weight measurements of broilers fed with three types of microalgae. IB: Broilers fed with *Tysochrysis lutea*, TB: Broilers fed with *Tetraselmis chuii*, PB: Broilers fed with *Porphyridium cruentum*. (*) shows that the group is significantly different than the control ($p<0.05$), (■) than the IB group.

4.1.1. Daily weight gain

The data of weight gain per day in the experimental groups are shown in Figure 5. It can be observed that in the time lapse covering the whole experiment (day 5 to 29) the only group with a higher weight gain ($p<0.05$) was TB, however, differences were also detected in other periods (day 5 to 7, day 14 to 21, and day 28 to 29).

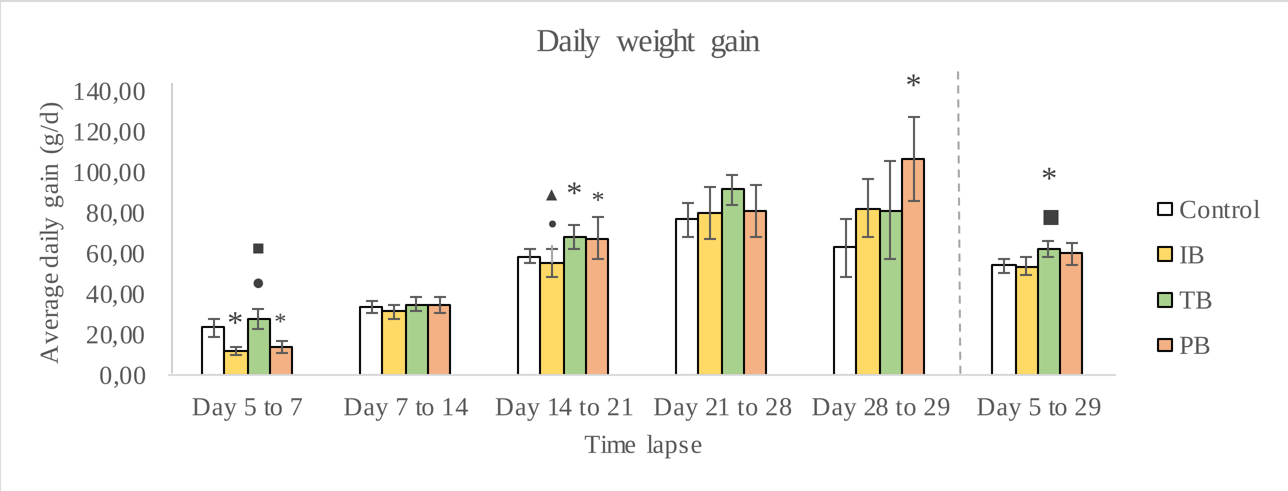

Figure 5: Average daily weight gain in the different experimental groups of chickens fed with three types of microalgae. IB: Broilers fed with *Tysochrysis lutea*, TB: Broilers fed with *Tetraselmis chuii*, PB: Broilers fed with *Porphyridium cruentum*.
(*) shows that the data obtained from this group is different than the control ($p<0.05$), (■) than the IB group, (▲) than the TB group, (●) than the PB group.

4.1.2. Daily feed intake

The data obtained on feed consumption are shown in Figure 6. During the time span covering the entire experimental period (day 5 to 29) differences ($p<0.05$) in all groups with respect to the control were detected. It can be observed that the chicks that ingested the most were those belonging to the IB and TB groups, while those that ate the least were the ones belonging to the PB group.

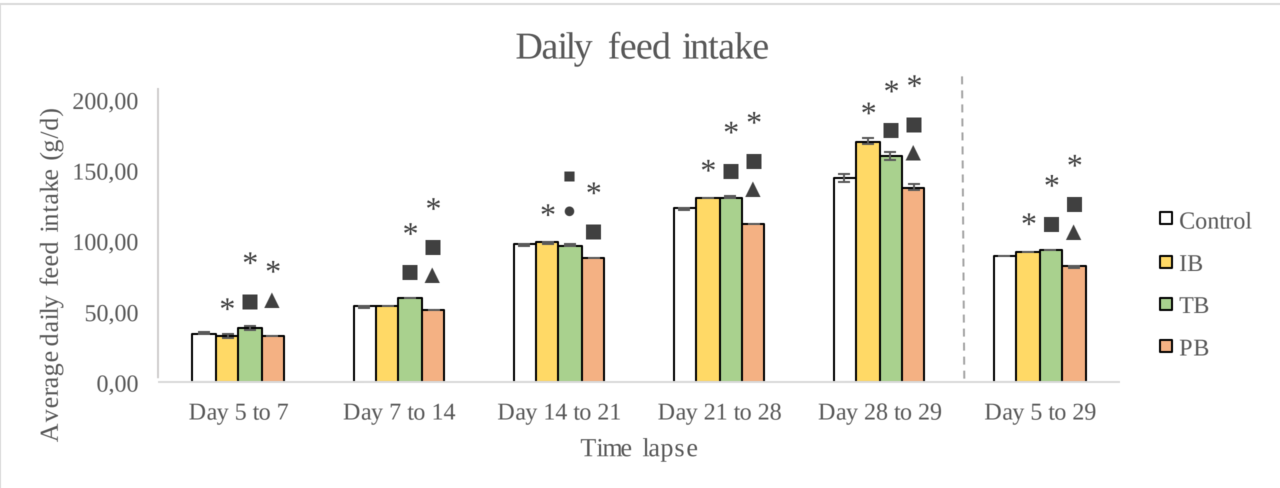

Figure 6: Daily feed intake of broilers supplied with three types of microalgae. IB: Broilers fed with *Tysochrysis lutea*, TB: Broilers fed with *Tetraselmis chuii*, PB: Broilers fed with *Porphyridium cruentum*.
(*) shows that the data obtained from this group is different than the control ($p<0.05$), (■) than the IB group, (▲) than the TB group, (●) than the PB group.

4.1.3. Feed conversion ratio

The feed conversion ratio data are shown in Figure 7. During the time span covering the entire experiment (day 5 to 29), differences were detected ($p<0.05$) between broilers belonging to the control group and the PB group, indicating that a higher percentage of this feed is converted into net mass in the birds. In addition, significant differences could also be found in the different time lapses between chicks fed with the different groups of microalgae.

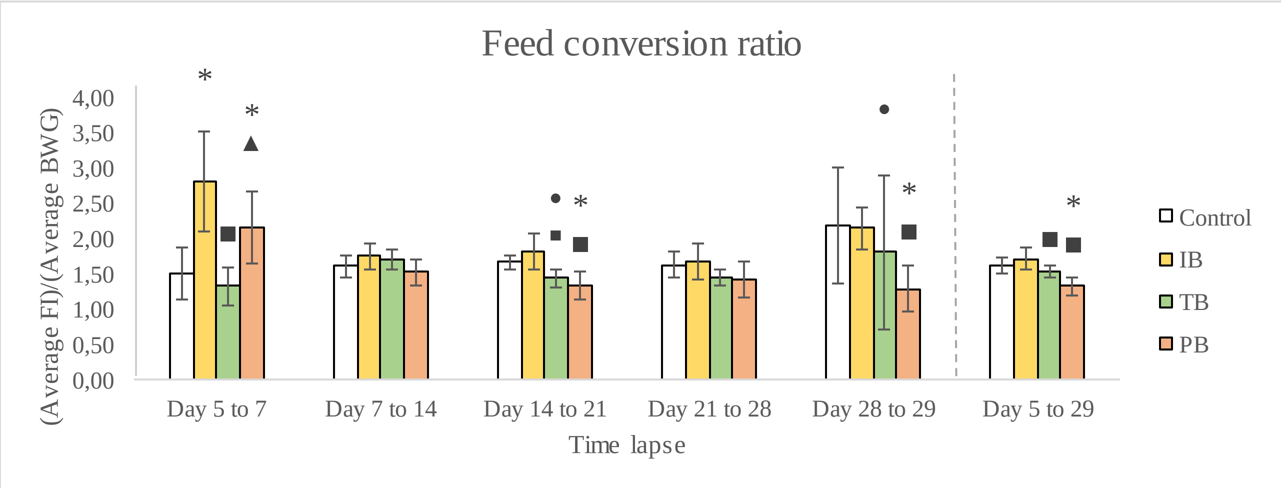

Figure 7: Feed conversion ratio in broilers fed with three types of microalgae. IB: Broilers fed with *Tysochrysis lutea*, TB: Broilers fed with *Tetraselmis chuii*, PB: Broilers fed with *Porphyridium cruentum*. (*) shows that the data obtained from this group is different than the control ($p<0.05$), (■) than the IB group, (▲) than the TB group, (●) than the PB group.

4.2. Gut Morphometry

Figure 8 shows illustrative micrographs at 4x magnification of different sections of the small intestine (duodenum and ileum) of the broilers used during this experimentation. The images belonging to the control group showed short and thick villi, which had a limited development in their crypts. On the other hand, in the experimental groups the small intestine appeared healthier, their villi were taller and thinner, and their crypts had a higher size and activity with the presence of goblet cells. In the duodenum sections, the high and thin villi showed a serrated surface type, with widened tips (related to the multiplication of goblet cells). In the ileum sections, proliferation of the epithelium and differentiation of goblet cells could be observed.

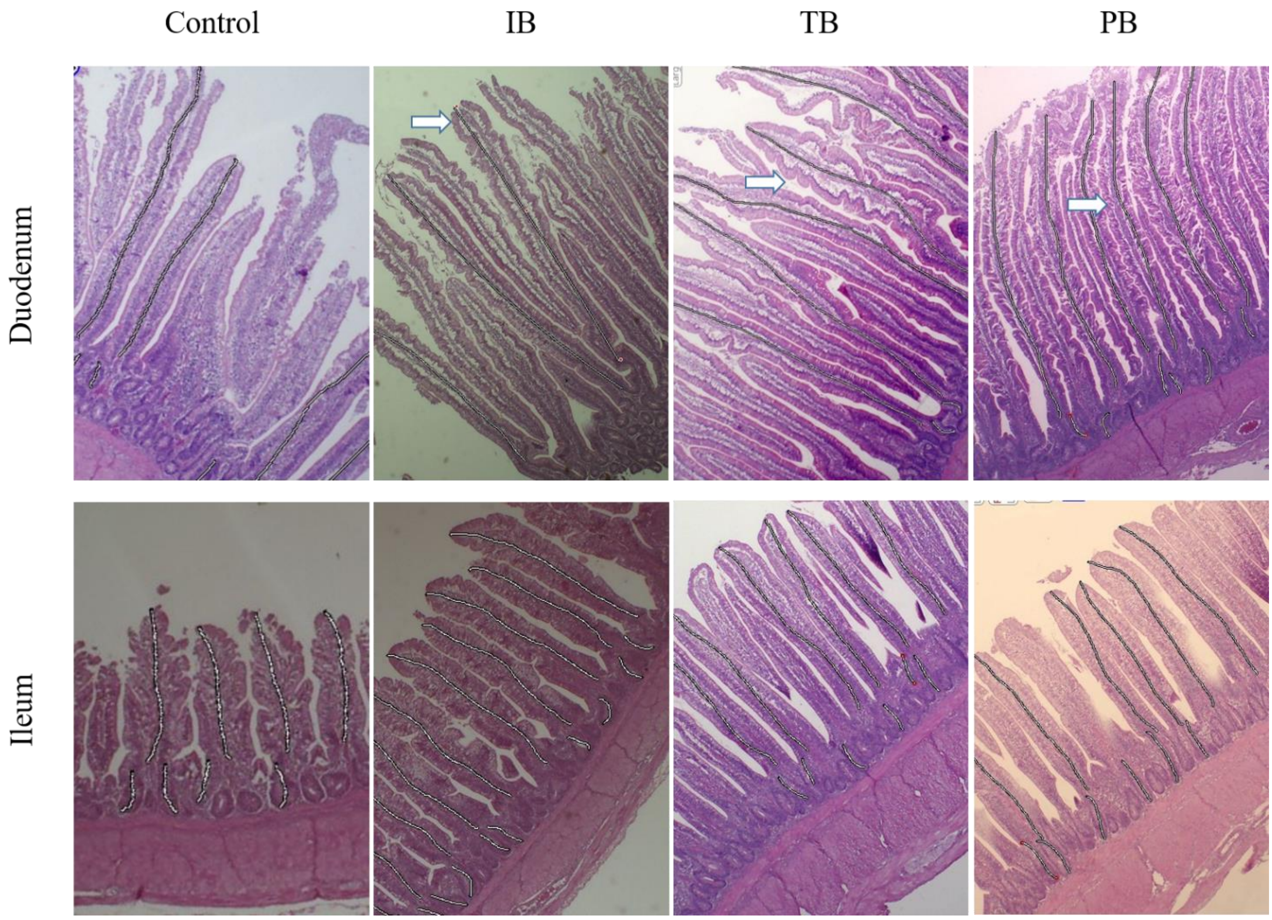

Figure 8: Illustrative images of duodenal and jejunal sections of birds fed with three types of microalgae. (4x magnification, hematoxylin and eosin staining). IB: Broilers fed with *Tysochrysis lutea*, TB: Broilers fed with *Tetraselmis chuii*, PB: Broilers fed with *Porphyridium cruentum*.
The drawn lines represent the length measured from the crypt to the apex of the villus. Birds belonging to groups IB, TB and PB have longer hairs compared to the control group. White arrows indicate sections of the duodenum of the experimental groups with more serrated surfaces, in contrast to the control group.

Pearson's *r* coefficient analysis showed a positive correlation between body weight and villus height to crypt depth ratio (0.977, $p<0.05$).

Table 2 summarizes the data obtained from the measurement of villus length, crypt depth, and goblet cell count in the duodenum. All broilers fed freeze-dried microalgae powder show improved parameters with regard to villus length and goblet cell count, with the PB group presenting the greatest difference. Regarding crypt depth, the IB and PB groups had larger crypt sizes than the control group. Villus height to crypt depth ratio was similar in all experimental groups.

Table 2: Morphometric characteristics of the duodenum in broilers fed with microalgae. IB: Broilers fed with *Tysochrysis lutea*, TB: Broilers fed with *Tetraselmis chuii*, PB: Broilers fed with *Porphyridium cruentum*.

Duodenum				
Parameter	Control	IB	TB	PB
Villus height (µm)	2659,1 ± 324,9	3233,92 ± 210,1 *	3454,45 ± 353,9 *	3842,46 ± 243,1 *■
[1]Crypt depth (µm)	357,3 (69,5)	404,8 (38,6) *	395,3 (52,8)	484,90 (48,7) *■▲
Villus height to crypt depth ratio	7,48 ± 1,0	8,15 ± 0,9	8,89 ± 1,0*	8,19 ± 0,9
Goblet cells count	20,8 ± 5,2	31,9 ± 4,9 *	35,7 ± 7,2 *	36,4 ± 4,0 *

(1) Values are medians plus their corresponding interquartile range (IQR).
Values are means ± SE (*N* = 9).
(*) shows that the group is significantly larger than the control ($p<0.05$), (■) than the IB group, (▲) than the TB group.

Table 3 summarizes the data obtained during the measurement of villus length, crypt depth, and goblet cell count in the ileum. Within this section, height of villi and villus height to crypt depth ratio were improved in the microalgae-fed groups compared to control conditions. Regarding crypt depth, the PB group was the only one that presented larger crypts than the control. No differences were found in goblet cell count.

Table 3: Morphometric characteristics of the ileum in broilers fed with three types of microalgae. IB: Broilers fed with *Tysochrysis lutea*, TB: Broilers fed with *Tetraselmis chuii*, PB: Broilers fed with *Porphyridium cruentum*.

Ileum				
Parameter	Control	IB	TB	PB
Villus height (µm)	1270,7 ± 143,7	1659,3 ± 228,6 *	1981,50 ± 117,4 *■	2303,9 ± 180,7 *■▲
Crypt depth (µm)	323,9 ± 29,4	362,4 ± 45,4	374,6 ± 54,06	406,9 ± 57,9 *
Villus height to crypt depth ratio	3,8 ± 0,6	4,2 ± 0,4 *	5,5 ± 0,8*	5,8 ± 0,8 *■
Goblet cells count	41,4 ± 4,5	46,6 ± 9,6	45,6 ± 3,5	52,9 ± 5,6 *

Values are means ± SE (*N* = 9).
(*) shows that the group is significantly larger than the control ($p<0.05$), (■) than the IB group, (▲) than the TB group.

4.3. Meat quality parameters

Regarding cooking loss, no differences were observed among any of the groups of broilers fed with microalgae. On the other hand, broilers belonging to the TB group had lower thawing loss compared to animals belonging to the control, IB and PB groups. The data obtained are described in Figure 9.

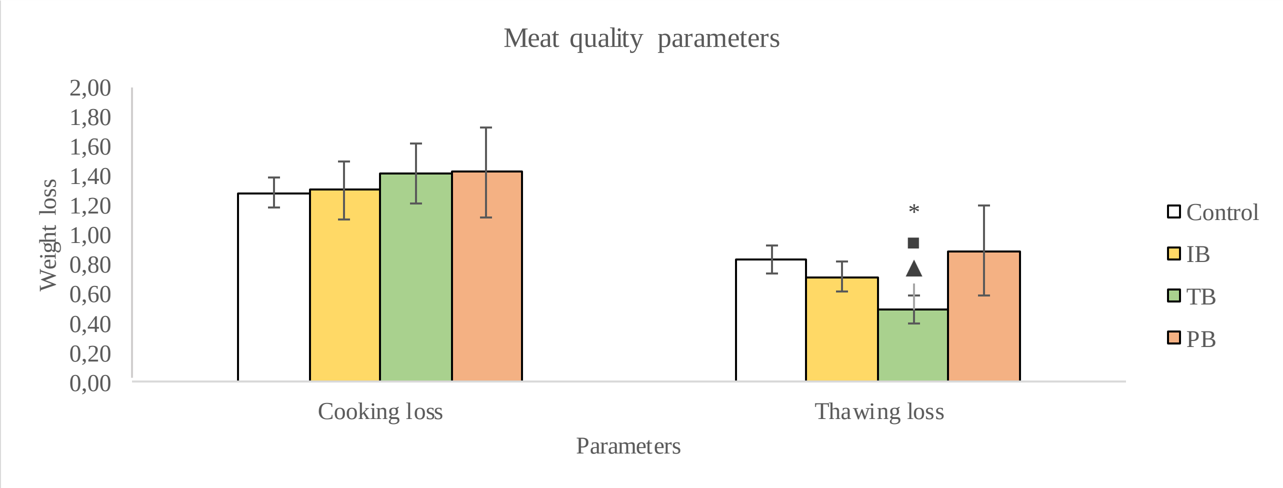

Figure 9: Meat quality parameters of the broilers. IB: Broilers fed with *Tysochrysis lutea*, TB: Broilers fed with *Tetraselmis chuii*, PB: Broilers fed with *Porphyridium cruentum.*
(*) shows that the group had lower losses than the control ($p<0.05$), (■) than the IB group, (▲) than the PB group.

5. Discussion

Feeding photosynthetic organisms to animals represent a great alternative to maximize growth performance and the immune response of some domesticated species, including broiler chickens (Abdelnour et al., 2019; Khan et al., 2021; Long et al., 2018). Although most studies demonstrating the efficacy of these organisms have been conducted using **macro**algae (Fries-Craft et al., 2021; Liu et al., 2021; Liu et al., 2020), recent research has demonstrated the ability of some microalgae species such as *Spirulina platensis*, *Chlorella vulgaris* and *Schizochytrium* sp. for improving various production parameters in poultry (Coudert et al., 2020; El-Bahr et al., 2020; Madeira et al., 2017; Yan & Kim, 2013). Microalgae are commonly used in aquaculture, as they serve as main sources of food for marine animals (Khatoon et al., 2021; W. Zhao et al., 2021). However, their application in the food industry related to non-aquatic animals still requires further research.

This investigation evaluated the capacity of the microalgae species *Tysochrysis lutea*, *Tetraselmis chuii* and *Porphyridium cruentum* as feed supplements in broiler diets. Information is scarce regarding the use of these species in poultry production. The results obtained are promising, especially in the case of *Porphyridium cruentum* and *Tetraselmis chuii*, which were able to improve growth performance, gut morphometry and thawing loss of broilers.

Tysochrysis lutea did not induce changes with regard to growth performance parameters of birds. These results are in agreement with those obtained with this microalgae in the diet of fish (Cardinaletti et al., 2018; Pulido-Rodriguez et al., 2021). It did not improve their production either.

Tetraselmis chuii induced positive effects regarding body weight gain and feed intake in broilers. Nonetheless, in terms of feed conversion ratio and morphometry parameters, it was surpassed by *Porphyridium cruentum*. These results corroborate those obtained from aquaculture studies (Khatoon et al., 2021; Zhao et al., 2021), where the efficacy of this microalgae in fish and shrimp feeding has also been demonstrated.

In addition, *Porphyridium cruentum* exerted positive results with regard to various parameters. In particular, the observed feed conversion ratio was the lowest, implying that a greater amount of feed is transformed into body weight in broilers. Birds fed with this microorganism presented the longest intestinal villi. Further, the experimental group corresponding to this microalga was the only one significantly higher in the measurement of crypt depths in the ileum, which has been associated with a quick regeneration of the villi (Bogucka et al., 2019; Saenphoom et al., 2013). It is important to highlight that the data obtained from morphometrical analyses were positively correlated with body weight increase.

Within this study, the biomass used for poultry feed was composed of fats, proteins and oligosaccharides. Some of the fatty acids and proteins present in *Tetraselmis chuii* are hexadecatetraenoic acid (C16:4ω-3), stearidonic acid (C18:4ω-3), and oleic acid (C18:1ω-9) (Lima et al., 2021); also, xanthophylls such as lutein and zeaxanthin along with β-carotene (Lewis & McCourt, 2004; Lima et al., 2021). Similarly, *Porphyridium cruentum* accumulates ω-3 fatty acids such as docosahexaenoic acid (C22:ω-6) and eicosapentaenoic acid (C22:ω-5) (Asgharpour et al., 2015); also, this species contains β-carotene, lutein, zeaxanthin and phycobilins which are responsible for their red coloration (Lima et al., 2021; Wehr et al., 2015). It has been shown that ω-3 fatty acids can improve intestinal morphometry in poultry (Wang et al., 2021) and are known for their high nutritional value in poultry production (Alagawany et al., 2019). On the other hand, compounds such as lutein, zeaxanthin and β-carotene are able to increase villi in the small intestine of broilers (Csernus et al., 2020) and enhance intestinal absorption of nutrients by enlarging the surface area of villi (Lokaewmanee et al., 2013). Phycobilins exert growth-promoting effects and improve intestinal morphology in broilers (Paul et al., 2021). As for oligosaccharides, they are able to improve intestinal structures (Cheng et al., 2019; Shang et al., 2015). *Porphyridium* sp. synthesizes significant amounts of structural polysaccharides and exopolysaccharides which induce morphological modifications in the small intestine and increase the amount of goblet cells in the mucosal layer of rats (Dvir et al., 2000). However, it must be taken into account that monogastric animals cannot digest these oligosaccharides so, inside the colon, they are fermented by a diverse group of microorganisms (Gomes & Malcata, 1999). The results obtained in this study may be related to the compounds previously mentioned, which stimulate the intestinal parameters and growth of the birds.

Concerning the immune system of broilers, it is known that the oligosaccharide side chains of mucins interact with bacterial adhesins preventing them from reaching the epithelium and damaging it (Cornick et al., 2015). This is partly due to the action of goblet cells, which line the villi and synthesize mucin, Muc 2, which is one of the major components of mucus (Johansson & Hansson, 2016). Mucus plays a critical role in nutrient transport, lubrication and protection within the intestinal epithelium (Baurhoo et al., 2007). The microalgae used in this experimentation were able to promote differentiation of goblet cells. This is particularly important because compounds such as plant extracts, probiotics and prebiotics, which have been shown to be beneficial for intestinal histology (Amer et al., 2021; Hashem et al., 2020; Lourenço et al., 2015; Omar et al., 2020; Xie et al., 2019), also promote goblet cell differentiation. Therefore, it is suggested that the use of microalgae as a feed additive may be relevant against bacterial infections, as they could reduce the negative impact of pathogenic bacteria and improve the health of broilers.

It has been suggested that a biomass incorporation rate of 1-5% is needed for a feed treatment to be effective. This could not considered suitable for mass use due to costs of production (Coudert et al., 2020). The amount of microalgae biomass used appears to influence the extent of the effects. The results obtained in this experiment showed that the implementation of lower doses of these microorganisms can also result in body weight gain (16% and 13% more weight with respect to the control in the case of *Tetraselmis chuii* and *Porphyridium cruentum* respectively, using 0.2% of biomass). These data corroborate previous results obtained using *C. vulgaris*, *A. coffeaformis* and *Chlorella* sp. (El-Bahr et al., 2020; Kang et al., 2013).

Data obtained on meat quality parameters indicated that the microalgae used in this study did not modify cooking loss. Nevertheless, the incorporation of *Tetraselmis chuii* reduced the thawing loss compared to the group of broilers fed the basal diet (Control). The results obtained are similar to those presented in the study by Khan et al. (2021), where microalgae-derived product reduced thawing loss without influencing cooking loss. Other study using *Spirulina platensis*, *Chlorella vulgaris* and *Amphora coffeaformis* reduced cooking loss, but did not alter thawing loss (El-Bahr et al., 2020). Although the results seem to be related to the species of microalgae used, the administration of these microorganisms in the broiler diet does not seem to have a negative impact on the parameters screened.

6.Conclusions

The inclusion of microalgae will not always result in positive outcomes in broiler growth performance or gut morphometry. Nevertheless, microalgae represent a new area of relevance for animal production, especially in terms of nutrition and health, so pursuing this field of research could generate important benefits in the future.

The results obtained with the microalgae species *Tetraselmis chuii* and *Porphyridium cruentum* demonstrate that these microorganisms could serve as potential feed additives in avian nutrition. The use of these species in small proportions (0.2% of the total feed) could generate positive effects with regard to growth performance. This improvement was related to the observed intestinal architecture and epithelial barrier. In addition, *Tetraselmis. chuii* administration improve thawing loss conditions in breast fillets. Further research is needed to improve the knowledge on the properties of microalgae, which may present new beneficial effects on both animal and human health.

7.Literature Cited

Abdelnour, S. A., Abd El-Hack, M. E., Arif, M., Khafaga, A. F., & Taha, A. E. (2019). The application of the microalgae *Chlorella spp*. As a supplement in broiler feed. *World's Poultry Science Journal*, *75*(2), 305–318. https://doi.org/10.1017/S0043933919000047

Abdollahi, M. R., Zaefarian, F., & Ravindran, V. (2018). Feed intake response of broilers: Impact of feed processing. *Animal Feed Science and Technology*, *237*, 154–165. https://doi.org/10.1016/j.anifeedsci.2018.01.013

Agostini, P. S., Solà-Oriol, D., Nofrarías, M., Barroeta, A. C., Gasa, J., & Manzanilla, E. G. (2012). Role of in-feed clove supplementation on growth performance, intestinal microbiology, and morphology in broiler chicken. *Livestock Science*, *147*(1–3), 113–118. https://doi.org/10.1016/j.livsci.2012.04.010

Alagawany, M., Elnesr, S. S., Farag, M. R., Abd El-Hack, M. E., Khafaga, A. F., Taha, A. E., Tiwari, R., Iqbal Yatoo, M., Bhatt, P., Khurana, S. K., & Dhama, K. (2019). Omega-3 and omega-6 fatty acids in poultry nutrition: Effect on production performance and health. *Animals*, *9*(8). https://doi.org/10.3390/ani9080573

Alders, R., Costa, R., Gallardo, R. A., Sparks, N., & Zhou, H. (2018). Smallholder poultry: Leveraging for sustainable food and nutrition security. In *Encyclopedia of Food Security and Sustainability* (Vol. 3). Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.21544-8

Alkhamis, Y., & Qin, J. G. (2016). Comparison of pigment and proximate compositions of *Tisochrysis lutea* in phototrophic and mixotrophic cultures. *Journal of Applied Phycology*, *28*(1), 35–42. https://doi.org/10.1007/s10811-015-0599-0

Amad, A. A., Männer, K., Wendler, K. R., Neumann, K., & Zentek, J. (2011). Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poultry Science*, *90*(12), 2811–2816. https://doi.org/10.3382/ps.2011-01515

Amer, S. A., Mohamed, W. A. M., Gharib, H. S. A., Al-Gabri, N. A., Gouda, A., Elabbasy, M. T., Abd El-Rahman, G. I., & Omar, A. E. (2021). Changes in the growth, ileal digestibility, intestinal histology, behavior, fatty acid composition of the breast muscles, and blood biochemical parameters of broiler chickens by dietary inclusion of safflower oil and vitamin C. *BMC Veterinary Research*, *17*(1), 68. https://doi.org/10.1186/s12917-021-02773-5

Asgharpour, M., Rodgers, B., & Hestekin, J. A. (2015). Eicosapentaenoic acid from *Porphyridium cruentum*: Increasing growth and productivity of microalgae for pharmaceutical products. *Energies*, *8*(9), 10487–10503. https://doi.org/10.3390/en80910487

Aviagen. (2018). *Ross Broiler guide*. https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-Broiler-Pocket-Guide-2020-EN.pdf

Barbeito, C. G. (2014). Histología de las aves. In N. González & C. G. Barbeito (Eds.), *Histología de las aves* (1st ed.). Universidad Nacional de La Plata – Editorial de la Universidad de La Plata. https://doi.org/10.35537/10915/43129

Baurhoo, B., Phillip, L., & Ruiz-Feria, C. A. (2007). Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. *Poultry Science*, *86*(6), 1070–1078. https://doi.org/10.1093/ps/86.6.1070

Bogucka, J., Ribeiro, D. M., Bogusławska-Tryk, M., Dankowiakowska, A., da Costa, R. P. R., & Bednarczyk, M. (2019). Microstructure of the small intestine in broiler chickens fed a diet with probiotic or synbiotic supplementation. *Journal of Animal Physiology and Animal Nutrition*, *103*(6), 1785–1791. https://doi.org/10.1111/jpn.13182

Bonilla-Ahumada, F. de J., Khandual, S., & Lugo-Cervantes, E. del C. (2018). Microencapsulation of algal biomass *Tetraselmis chuii* by spray-drying using different encapsulation materials for better preservation of beta-carotene and antioxidant compounds. *Algal Research*, *36*(October), 229–238. https://doi.org/10.1016/j.algal.2018.10.006

Bregendahl, K., Sell, J. L., & Zimmerman, D. R. (2002). Effect of low-protein diets on growth performance and body composition of broiler chicks. *Poultry Science*, *81*(8), 1156–1167. https://doi.org/10.1093/ps/81.8.1156

Cardinaletti, G., Messina, M., Bruno, M., Tulli, F., Poli, B. M., Giorgi, G., Chini-Zittelli, G., Tredici, M., & Tibaldi, E. (2018). Effects of graded levels of a blend of *Tisochrysis lutea* and *Tetraselmis suecica* dried biomass on growth and muscle tissue composition of European sea bass (*Dicentrarchus labrax*) fed diets low in fish meal and oil. *Aquaculture*, *485*(July 2017), 173–182. https://doi.org/10.1016/j.aquaculture.2017.11.049

Casas-Arrojo, V., Decara, J., Arrojo-Agudo, M. de los Á., Pérez-Manríquez, C., & Abdala-Díaz, R. T. (2021). Immunomodulatory, antioxidant activity and cytotoxic effect of sulfated polysaccharides from porphyridium cruentum. (s.f.gray) nägeli. *Biomolecules*, *11*(4). https://doi.org/10.3390/biom11040488

Cheng, Y. F., Chen, Y. P., Chen, R., Su, Y., Zhang, R. Q., He, Q. F., Wang, K., Wen, C., & Zhou, Y. M. (2019). Dietary mannan oligosaccharide ameliorates cyclic heat stress-induced damages on intestinal oxidative status and barrier integrity of broilers. *Poultry Science*, *98*(10), 4767–4776. https://doi.org/10.3382/ps/pez192

Cornick, S., Tawiah, A., & Chadee, K. (2015). Roles and regulation of the mucus barrier in the gut. *Tissue Barriers*, *3*(1), 1–2. https://doi.org/10.4161/21688370.2014.982426

Coudert, E., Baéza, E., & Berri, C. (2020). Use of algae in poultry production: a review. *World's Poultry Science Journal*, *76*(4), 767–786. https://doi.org/10.1080/00439339.2020.1830012

Cox, J. A., Vlieghe, E., Mendelson, M., Wertheim, H., Ndegwa, L., Villegas, M. V., Gould, I., & Levy Hara, G. (2017). Antibiotic stewardship in low- and middle-income countries: the same but different? *Clinical Microbiology and Infection*, *23*(11), 812–818. https://doi.org/10.1016/j.cmi.2017.07.010

Cramer, T. A., Kim, H. W., Chao, Y., Wang, W., Cheng, H. W., & Kim, Y. H. B. (2018). Effects of probiotic (*Bacillus subtilis*) supplementation on meat quality characteristics of breast muscle from broilers exposed to chronic heat stress. *Poultry Science*, *97*(9), 3358–3368. https://doi.org/10.3382/ps/pey176

Crespo, N., & Esteve-Garcia, E. (2002). Nutrient and fatty acid deposition in broilers fed different dietary fatty acid profiles. *Poultry Science*, *81*(10), 1533–1542. https://doi.org/10.1093/ps/81.10.1533

Csernus, B., Biro, S., & Babinszky, L. (2020). Effect of Carotenoids, Oligosaccharides and Anthocyanins on Growth Performance, Immunological Parameters and Intestinal Morphology in Broiler Chickens Challenged with *Escherichia coli* Lipopolysaccharide. *Animals, 347*. https://doi.org/10.3390/ani10020347

Dvir, I., Chayoth, R., Sod-Moriah, U., Shany, S., Nyska, A., Stark, A. H., Madar, Z., & Arad, S. M. (2000). Soluble polysaccharide and biomass of red microalga *Porphyridium sp*. alter intestinal morphology and reduce serum cholesterol in rats. *British Journal of Nutrition*, *84*(4), 469–476. https://doi.org/10.1017/s000711450000177x

El-Bahr, S., Shousha, S., Shehab, A., Khattab, W., Ahmed-Farid, O., Sabike, I., El-Garhy, O., Albokhadaim, I., & Albosadah, K. (2020). Effect of dietary microalgae on growth performance, profiles of amino and fatty acids, antioxidant status, and meat quality of broiler chickens. *Animals*, *10*(5), 1–14. https://doi.org/10.3390/ani10050761

Erol, H. B. U., Menegazzo, M. L., Sandefur, H., Gottberg, E., Vaden, J., Asgharpour, M., Hestekin, C. N., & Hestekin, J. A. (2020). *Porphyridium cruentum* grown in ultra-filtered swine wastewater and its effects on microalgae growth productivity and fatty acid composition. *Energies*, *13*(12). https://doi.org/10.3390/en13123194

Fletcher, D. L. (1999). Broiler breast meat color variation, pH, and texture. *Poultry Science*, *78*(9), 1323–1327. https://doi.org/10.1093/ps/78.9.1323

Fries-Craft, K., Meyer, M. M., & Bobeck, E. A. (2021). Algae-based feed ingredient protects intestinal health during Eimeria challenge and alters systemic immune responses with differential outcomes observed during acute feed restriction. *Poultry Science*, *100*(9), 101369. https://doi.org/10.1016/j.psj.2021.101369

Geresh, S., Arad, S. (Malis), Levy-Ontman, O., Zhang, W., Tekoah, Y., & Glaser, R. (2009). Isolation and characterization of poly- and oligosaccharides from the red microalga *Porphyridium sp*. *Carbohydrate Research*, *344*(3), 343–349. https://doi.org/10.1016/j.carres.2008.11.012

Gomes, A. M. P., & Malcata, F. X. (1999). *Bifidobacterium spp.* and *Lactobacillus acidophilus*: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends in Food Science and Technology*, *10*(4–5), 139–157. https://doi.org/10.1016/S0924-2244(99)00033-3

Gonçalves de Oliveira-Júnior, R., Grougnet, R., Bodet, P. E., Bonnet, A., Nicolau, E., Jebali, A., Rumin, J., & Picot, L. (2020). Updated pigment composition of *Tisochrysis lutea* and purification of fucoxanthin using centrifugal partition chromatography coupled to flash chromatography for the chemosensitization of melanoma cells. *Algal Research*, *51*(August), 102035. https://doi.org/10.1016/j.algal.2020.102035

Goodman, B. E. (2010). Insights into digestion and absorption of major nutrients in humans. *American Journal of Physiology - Advances in Physiology Education*, *34*(2), 44–53. https://doi.org/10.1152/advan.00094.2009

Gopi, M., Dutta, N., Kumar Pattanaik, A., Ekant Jadhav, S., Madhupriya, V., Kumar Tyagi, P., & Mohan, J. (2020). Effect of polyphenol extract on performance, serum biochemistry, skin pigmentation and carcass characteristics in broiler chickens fed with different cereal sources under hot-humid conditions. *Saudi Journal of Biological Sciences*, *27*(10), 2719–2726. https://doi.org/10.1016/j.sjbs.2020.06.021

Guiry, M. D. (2012). How many species of algae are there? *Journal of Phycology*, *48*(5), 1057–1063. https://doi.org/10.1111/j.1529-8817.2012.01222.x

Hashem, M. A., Neamat-Allah, A. N. F., Hammza, H. E. E., & Abou-Elnaga, H. M. (2020). Impact of dietary supplementation with *Echinacea purpurea* on growth performance, immunological, biochemical, and pathological findings in broiler chickens infected by pathogenic *E. coli*. *Tropical Animal Health and Production*, *52*(4), 1599–1607. https://doi.org/10.1007/s11250-019-02162-z

Hicks, M. H. (2020). Results of new FDA rules on antibiotic use in US food-producing animals. *Journal of Public Health (United Kingdom)*, *42*(4), E573–E574. https://doi.org/10.1093/pubmed/fdz174

Holdstock, J., Aalhus, J. L., Uttaro, B. A., López-Campos, Ó., Larsen, I. L., & Bruce, H. L. (2014). The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade. *Meat Science*, *98*(4), 842–849. https://doi.org/10.1016/j.meatsci.2014.07.029

Honikel, K. O., & Hamm, R. (1994). Measurement of water-holding capacity and juiciness.

Quality Attributes and Their Measurement in Meat, Poultry and Fish Products, 125–161. https://doi.org/10.1007/978-1-4615-2167-9_5

Honikel, Karl O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, *49*(4), 447–457. https://doi.org/10.1016/S0309-1740(98)00034-5

Hu, H., Wang, H. F., Ma, L. L., Shen, X. F., & Zeng, R. J. (2018). Effects of nitrogen and phosphorous stress on the formation of high value LC-PUFAs in *Porphyridium cruentum*. *Applied Microbiology and Biotechnology*, *102*(13), 5763–5773. https://doi.org/10.1007/s00253-018-8943-3

Javed, M., Bilal, M. J., Mehmood, M., Ashraf, M., Waqar, A., Saeed, M., & Nashat, N. (2019). *Microalgae as a Feedstock for Biofuel Production: Current Status and Future Prospects* (pp. 1–39).

Johansson, M. E. V., & Hansson, G. C. (2016). Immunological aspects of intestinal mucus and mucins. *Nature Reviews Immunology*, *16*(10), 639–649. https://doi.org/10.1038/nri.2016.88

Kabir, S. M. L., Rahman, M. M., Rahman, M. B., Rahman, M. M., & Ahmed, S. U. (2004). The dynamics of probiotics on growth performance and immune response in broilers. *International Journal of Poultry Science*, *3*(5), 361–364. https://doi.org/10.3923/ijps.2004.361.364

Kang, H. K., Salim, H. M., Akter, N., Kim, D. W., Kim, J. H., Bang, H. T., Kim, M. J., Na, J. C., Hwangbo, J., Choi, H. C., & Suh, O. S. (2013). Effect of various forms of dietary *Chlorella* supplementation on growth performance, immune characteristics, and intestinal microflora population of broiler chickens. *Journal of Applied Poultry Research*, *22*(1), 100–108. https://doi.org/10.3382/japr.2012-00622

Karcher, D. M., & Mench, J. A. (2017). Overview of commercial poultry production systems and their main welfare challenges. In *Advances in Poultry Welfare*. Elsevier Ltd. https://doi.org/10.1016/B978-0-08-100915-4.00001-4

Khan, I. A., Parker, N. B., Löhr, C. V., & Cherian, G. (2021). Docosahexaenoic acid (22:6 n-3)-rich microalgae along with methionine supplementation in broiler chickens: effects on production performance, breast muscle quality attributes, lipid profile, and incidence of white striping and myopathy. *Poultry Science*, *100*(2), 865–874. https://doi.org/10.1016/j.psj.2020.10.069

Khatoon, H., Penz, K. P., Banerjee, S., Mahmud, A. I., Rahman, M. R., Mian, S., Minhaz, T. M., & Hossain, S. (2021). Improvement of water quality, survivality, growth performance, and proximate composition of Penaeus monodon postlarvae through immobilizing *Tetraselmis chuii*. *Bioresource Technology Reports*, *15*(June), 100755. https://doi.org/10.1016/j.biteb.2021.100755

Knoop, K. A., & Newberry, R. D. (2018). Goblet cells: multifaceted players in immunity at mucosal surfaces. *Mucosal Immunology*, *11*(6), 1551–1557. https://doi.org/10.1038/s41385-018-0039-y

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell*, *165*(6), 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041

Koutsos, E. A., Calvert, C. C., & Klasing, K. C. (2003). The effect of an acute phase response on tissue carotenoid levels of growing chickens (*Gallus gallus domesticus*). *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *135*(4), 635–646. https://doi.org/10.1016/S1095-6433(03)00158-2

Kumar, D., Pornsukarom, S., & Thakur, S. (2019). Antibiotic Usage in Poultry Production and Antimicrobial-Resistant Salmonella in Poultry. *Food Safety in Poultry Meat Production*, 47–66. https://doi.org/10.1007/978-3-030-05011-5_3

Larson, G., & Fuller, D. Q. (2014). The evolution of animal domestication. *Annual Review of Ecology, Evolution, and Systematics*, *45*(September), 115–136. https://doi.org/10.1146/annurev-ecolsys-110512-135813

Levasseur, W., Perré, P., & Pozzobon, V. (2020). A review of high value-added molecules production by microalgae in light of the classification. *Biotechnology Advances*, *41*(January), 107545. https://doi.org/10.1016/j.biotechadv.2020.107545

Lewis, L. A., & McCourt, R. M. (2004). Green algae and the origin of land plants. *American Journal of Botany*, *91*(10), 1535–1556. https://doi.org/10.3732/ajb.91.10.1535

Li, W., Li, W., Liu, R., Zheng, M., Feng, F., Liu, D., Guo, Y., Zhao, G., & Wen, J. (2020). New insights into the associations among feed efficiency, metabolizable efficiency traits and related QTL regions in broiler chickens. *Journal of Animal Science and Biotechnology*, *11*(1). https://doi.org/10.1186/s40104-020-00469-8

Lima, S., Schulze, P. S. C., Schüler, L. M., Rautenberger, R., Morales-Sánchez, D., Santos, T. F., Pereira, H., Varela, J. C. S., Scargiali, F., Wijffels, R. H., & Kiron, V. (2021). Flashing light emitting diodes (LEDs) induce proteins, polyunsaturated fatty acids and pigments in three microalgae. *Journal of Biotechnology*, *325*, 15–24. https://doi.org/10.1016/j.jbiotec.2020.11.019

Lin, J. (2014). Antibiotic growth promoters enhance animal production by targeting intestinal bile salt hydrolase and its producers. *Frontiers in Microbiology*, *5*(FEB), 1–4. https://doi.org/10.3389/fmicb.2014.00033

Lippens, M., Room, G., De Groote, G., & Decuypere, E. (2000). Early and temporary quantitative food restriction of broiler chickens. 1. Effects on performance characteristics, mortality and meat quality. *British Poultry Science*, *41*(3), 343–354.

https://doi.org/10.1080/713654926

Liu, K., Jia, M., & Wong, E. A. (2020). Delayed access to feed affects broiler small intestinal morphology and goblet cell ontogeny. *Poultry Science*, *99*(11), 5275–5285. https://doi.org/10.1016/j.psj.2020.07.040

Liu, T., Wang, C., Wu, X., Ren, M., Hu, Q., Jin, E., & Gu, Y. (2021). Effect of Boron on Microstructure, Immune Function, Expression of Tight Junction Protein, Cell Proliferation and Apoptosis of Duodenum in Rats. *Biological Trace Element Research*, *199*(1), 205–215. https://doi.org/10.1007/s12011-020-02123-w

Liu, W. C., Guo, Y., Zhao, Z. H., Jha, R., & Balasubramanian, B. (2020). Algae-Derived Polysaccharides Promote Growth Performance by Improving Antioxidant Capacity and Intestinal Barrier Function in Broiler Chickens. *Frontiers in Veterinary Science*, *7*(December), 0–4. https://doi.org/10.3389/fvets.2020.601336

Liu, W. C., Zhu, Y. R., Zhao, Z. H., Jiang, P., & Yin, F. Q. (2021). Effects of dietary supplementation of algae-derived polysaccharides on morphology, tight junctions, antioxidant capacity and immune response of duodenum in broilers under heat stress. *Animals*, *11*(8). https://doi.org/10.3390/ani11082279

Lokaewmanee, K., Yamauchi, K., & Okuda, N. (2013). Effects of dietary red pepper on egg yolk colour and histological intestinal morphology in laying hens. *Journal of Animal Physiology and Animal Nutrition*, *97*(5), 986–995. https://doi.org/10.1111/jpn.12011

Long, S. F., Kang, S., Wang, Q. Q., Xu, Y. T., Pan, L., Hu, J. X., Li, M., & Piao, X. S. (2018). Dietary supplementation with DHA-rich microalgae improves performance, serum composition, carcass trait, antioxidant status, and fatty acid profile of broilers. *Poultry Science*, *97*(6), 1881–1890. https://doi.org/10.3382/ps/pey027

Lourenço, M. C., Kuritza, L. N., Hayashi, R. M., Miglino, L. B., Durau, J. F., Pickler, L., & Santin, E. (2015). Effect of a mannanoligosaccharide-supplemented diet on intestinal mucosa T lymphocyte populations in chickens challenged with Salmonella Enteritidis. *Journal of Applied Poultry Research*, *24*(1), 15–22. https://doi.org/10.3382/japr/pfu002

Lovejoy, C. (2020). Plankton of the Open Arctic Ocean. In *Encyclopedia of the World's Biomes* (Issue 200 m). Elsevier Inc. https://doi.org/10.1016/b978-0-12-409548-9.11979-7

Lu, L., Wang, J., Yang, G., Zhu, B., & Pan, K. (2017). Heterotrophic growth and nutrient productivities of *Tetraselmis chuii* using glucose as a carbon source under different C/N ratios. *Journal of Applied Phycology*, *29*(1), 15–21. https://doi.org/10.1007/s10811-016-0919-z

Madeira, M. S., Cardoso, C., Lopes, P. A., Coelho, D., Afonso, C., Bandarra, N. M., & Prates, J. A. M. (2017). Microalgae as feed ingredients for livestock production and meat quality: A review. *Livestock Science*, *205*, 111–121. https://doi.org/10.1016/j.livsci.2017.09.020

Maltsev, Y., & Maltseva, K. (2021). Fatty acids of microalgae: diversity and applications. In *Reviews in Environmental Science and Biotechnology* (Vol. 20, Issue 2). Springer Netherlands. https://doi.org/10.1007/s11157-021-09571-3

Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: Impacts on human health. *Clinical Microbiology Reviews*, *24*(4), 718–733. https://doi.org/10.1128/CMR.00002-11

Matos, J., Cardoso, C., Gomes, A., Campos, A. M., Falé, P., Afonso, C., & Bandarra, N. M. (2019). Bioprospection of: *Isochrysis galbana* and its potential as a nutraceutical. *Food and Function*, *10*(11), 7333–7342. https://doi.org/10.1039/c9fo01364d

Miezeliene, A., Alencikiene, G., Gruzauskas, R., & Barstys, T. (2011). The effect of dietary selenium supplementation on meat quality of broiler chickens. *Biotechnology, Agronomy and Society and Environment*, *15*(SPEC. ISSUE 1), 61–69.

Miller, R. K. (2002). Factors affecting the quality of raw meat. In *Meat Processing*. Woodhead Publishing Limited. https://doi.org/10.1533/9781855736665.1.27

Millet, S., & Maertens, L. (2011). The European ban on antibiotic growth promoters in animal feed: From challenges to opportunities. *Veterinary Journal*, *187*(2), 143–144. https://doi.org/10.1016/j.tvjl.2010.05.001

Nain, S., Renema, R. A., Zuidhof, M. J., & Korver, D. R. (2012). Effect of metabolic efficiency and intestinal morphology on variability in n-3 polyunsaturated fatty acid enrichment of eggs. *Poultry Science*, *91*(4), 888–898. https://doi.org/10.3382/ps.2011-01661

Obajuluwa, O. V., Sanwo, K. A., Egbeyale, L. T., & Fafiolu, A. O. (2020). Performance, blood profile and gut morphometry of broiler chickens fed diets supplemented with Yohimbe (*Pausynistalia yohimbe*) and Larvacide. *Veterinary and Animal Science*, *10*(June), 100127. https://doi.org/10.1016/j.vas.2020.100127

Odetallah, N. H., Wang, J. J., Garlich, J. D., & Shih, J. C. H. (2005). Versazyme supplementation of broiler diets improves market growth performance. *Poultry Science*, *84*(6), 858–864. https://doi.org/10.1093/ps/84.6.858

Omar, A. E., Al-Khalaifah, H. S., Mohamed, W. A. M., Gharib, H. S. A., Osman, A., Al-Gabri, N. A., & Amer, S. A. (2020). Effects of Phenolic-Rich Onion (*Allium cepa L.*) Extract on the Growth Performance, Behavior, Intestinal Histology, Amino Acid Digestibility, Antioxidant Activity, and the Immune Status of Broiler Chickens. *Frontiers in Veterinary Science*, *7*(November), 1–14. https://doi.org/10.3389/fvets.2020.582612

Patel, A. K., Singhania, R. R., Awasthi, M. K., Varjani, S., Bhatia, S. K., Tsai, M. L., Hsieh, S. L., Chen, C. W., & Dong, C. Di. (2021). Emerging prospects of macro- and microalgae as prebiotic. *Microbial Cell Factories*, *20*(1), 1–16. https://doi.org/10.1186/s12934-021-01601-7

Patil, V., Källqvist, T., Olsen, E., Vogt, G., & Gislerød, H. R. (2007). Fatty acid composition of 12 microalgae for possible use in aquaculture feed. *Aquaculture International*, *15*(1), 1–9. https://doi.org/10.1007/s10499-006-9060-3

Paul, S. S., Vantharam Venkata, H. G. R., Raju, M. V. L. N., Rama Rao, S. V., Nori, S. S., Suryanarayan, S., Kumar, V., Perveen, Z., & Prasad, C. S. (2021). Dietary supplementation of extracts of red sea weed (*Kappaphycus alvarezii*) improves growth, intestinal morphology, expression of intestinal genes and immune responses in broiler chickens. *Journal of the Science of Food and Agriculture*, *101*(3), 997–1008. https://doi.org/10.1002/jsfa.10708

Pearce, S. C., Weber, G. J., Van Sambeek, D. M., Soares, J. W., Racicot, K., & Breault, D. T. (2020). Intestinal enteroids recapitulate the effects of short-chain fatty acids on the intestinal epithelium. *PLoS ONE*, *15*(4), 1–23. https://doi.org/10.1371/journal.pone.0230231

Piekarska, J., Mišta, D., Houszka, M., Króliczewska, B., Zawadzki, W., & Gorczykowski, M. (2011). *Trichinella spiralis*: The influence of short chain fatty acids on the proliferation of lymphocytes, the goblet cell count and apoptosis in the mouse intestine. *Experimental Parasitology*, *128*(4), 419–426. https://doi.org/10.1016/j.exppara.2011.05.019

Pulido-Rodriguez, L. F., Cardinaletti, G., Secci, G., Randazzo, B., Bruni, L., Cerri, R., Olivotto, I., Tibaldi, E., & Parisi, G. (2021). Appetite regulation, growth performances and fish quality are modulated by alternative dietary protein ingredients in gilthead sea bream (*Sparus aurata*) culture. *Animals*, *11*(7), 1–15. https://doi.org/10.3390/ani11071919

Rasdi, N. W., & Qin, J. G. (2015). Effect of N:P ratio on growth and chemical composition of *Nannochloropsis oculata* and *Tisochrysis lutea*. *Journal of Applied Phycology*, *27*(6), 2221–2230. https://doi.org/10.1007/s10811-014-0495-z

Riley, W. W., Nickerson, J. G., & Burton, G. W. (2021). Effect of oxidized β-carotene on the growth and feed efficiency of broilers. *Poultry Science*, *100*(6). https://doi.org/10.1016/j.psj.2021.101088

Saenphoom, P., Liang, J. B., Ho, Y. W., Loh, T. C., & Rosfarizan, M. (2013). Effects of enzyme treated palm kernel expeller on metabolizable energy, growth performance, villus height and digesta viscosity in broiler chickens. *Asian-Australasian Journal of Animal Sciences*, *26*(4), 537–544. https://doi.org/10.5713/ajas.2012.12463

Šefcová, M., Larrea-Álvarez, M., Larrea-Álvarez, C., Revajová, V., Karaffová, V., Koščová, J., Nemcová, R., Ortega-Paredes, D., Vinueza-Burgos, C., Levkut, M., & Herich, R. (2020). Effects of *Lactobacillus Fermentum* supplementation on body weight and pro-inflammatory cytokine expression in Campylobacter Jejuni-challenged chickens. *Veterinary Sciences*, *7*(3). https://doi.org/10.3390/vetsci7030121

Shang, Y., Regassa, A., Kim, J. H., & Kim, W. K. (2015). The effect of dietary fructooligosaccharide supplementation on growth performance, intestinal morphology, and immune responses in broiler chickens challenged with *Salmonella Enteritidis* lipopolysaccharides. *Poultry Science*, *94*(12), 2887–2897. https://doi.org/10.3382/ps/pev275

Vale, M. A., Ferreira, A., Pires, J. C. M., & Gonçalves, A. L. (2020). CO2 capture using microalgae. *Advances in Carbon Capture*, 381–405. https://doi.org/10.1016/b978-0-12-819657-1.00017-7

Wafaa, E.-G. (2020). Microalgae in Poultry Field: A Comprehensive Perspectives. *Advances in Animal and Veterinary Sciences*, *8*(9), 888–897. https://doi.org/http://dx.doi.org/10.17582/journal.aavs/2020/8.9.888.897

Wang, J., Clark, D. L., Jacobi, S. K., & Velleman, S. G. (2021). Supplementation of vitamin E and omega-3 fatty acids during the early posthatch period on intestinal morphology and gene expression differentiation in broilers. *Poultry Science*, *100*(3), 100954. https://doi.org/10.1016/j.psj.2020.12.051

Wehr, J., Sheath, R., & Kociolek, P. (2015). *Freshwater Algae of North America: Ecology and Classification*.

Wen, C., Yan, W., Zheng, J., Ji, C., Zhang, D., Sun, C., & Yang, N. (2018). Feed efficiency measures and their relationships with production and meat quality traits in slower growing broilers. *Poultry Science*, *97*(7), 2356–2364. https://doi.org/10.3382/ps/pey062

Wilson, F. D., Cummings, T. S., Barbosa, T. M., Williams, C. J., Gerard, P. D., & Peebles, E. D. (2018). Comparison of two methods for determination of intestinal villus to crypt ratios and documentation of early age-associated ratio changes in broiler chickens1,2,3. *Poultry Science*, *97*(5), 1757–1761. https://doi.org/10.3382/ps/pex349

Xie, S., Zhao, S., Jiang, L., Lu, L., Yang, Q., & Yu, Q. (2019). *Lactobacillus reuteri* Stimulates Intestinal Epithelial Proliferation and Induces Differentiation into Goblet Cells in Young Chickens. *Journal of Agricultural and Food Chemistry*, *67*(49), 13758–13766. https://doi.org/10.1021/acs.jafc.9b06256

Xu, Y., Tian, Y., Cao, Y., Li, J., Guo, H., Su, Y., Tian, Y., Wang, C., Wang, T., & Zhang, L. (2019). Probiotic properties of *Lactobacillus paracasei* subsp. Paracasei L1 and its growth performance-promotion in chicken by improving the intestinal microflora. *Frontiers in Physiology*, *10*(JUL). https://doi.org/10.3389/fphys.2019.00937

Yan, L., & Kim, I. H. (2013). Effects of dietary ω -3 fatty acid-enriched microalgae supplementation on growth performance, blood profiles, meat quality, and fatty acid composition of meat in broilers. *Journal of Applied Animal Research*, *41*(4), 392–397. https://doi.org/10.1080/09712119.2013.787361

Zhang, H., Li, D., Liu, L., Liu, Y., Xu, L., Zhu, M., & He, X. (2019). Cellular composition and differentiation signaling in chicken small intestinal epithelium. *Animals*, *9*(11), 1–12. https://doi.org/10.3390/ani9110870

Zhao, W., Fang, H. H., Liu, Z. Z., Chen, J. M., Zhang, C. W., Gao, B. Y., & Niu, J. (2021). Responses in growth performance, enzymatic activity, immune function and liver health after dietary supplementation of *Porphyridium sp*. in juvenile golden pompano (*Trachinotus ovatus*). *Aquaculture Nutrition*, *27*(3), 679–690. https://doi.org/10.1111/anu.13214

Zhao, Y., Zeng, D., Wang, H., Qing, X., Sun, N., Xin, J., Luo, M., Khalique, A., Pan, K., Shu, G., Jing, B., & Ni, X. (2020). Dietary Probiotic *Bacillus licheniformis H2* Enhanced Growth Performance, Morphology of Small Intestine and Liver, and Antioxidant Capacity of Broiler Chickens Against *Clostridium perfringens*–Induced Subclinical Necrotic Enteritis. *Probiotics and Antimicrobial Proteins*, *12*(3), 883–895. https://doi.org/10.1007/s12602-019-09597-8

Zhu, N., Wang, J., Yu, L., Zhang, Q., Chen, K., & Liu, B. (2019). Modulation of growth performance and intestinal microbiota in chickens fed plant extracts or virginiamycin. *Frontiers in Microbiology*, *10*(JUN), 1–16. https://doi.org/10.3389/fmicb.2019.01333