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## Using chlorophyll extract as a natural food source for *Carassius auratus* and adult male Wistar Hannover rats

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### Abstract

Chlorophyll is found in plants everywhere and it is a natural biological energy source. Because seaweed and spinach (*Spinacia oleracea* L.) are highly concentrated with chlorophyll, this study evaluated the effect of chlorophyll extracted from seaweeds and *S. oleracea* as a supplementary food source for goldfish (*Carassius auratus*) and adult male Wistar Hannover rats. Our results showed significant body weight gain in *C. auratus* treated with chlorophyll extracted from seaweeds; *C. auratus* also showed a brighter and more vibrant color. Our study also showed that chlorophyll extracted from *S. oleracea* caused a significant increase in the cholesterol, TG, and HDL levels in the treated rats compared to the control group. These results indicate that chlorophyll could be a more suitable alternative for feeding *C. auratus* than commercial foods, and an efficacious agent to improve the rise of lipid and cholesterol levels in people with vascular conditions.

**Keywords:** Chlorophyll, Goldfish, *Carassius auratus*, Seaweeds, Spinach, *S. oleracea*

### Introduction

Plants contain chlorophyll, which is an essential component of a wide variety of foods, including green leafy vegetables, wheatgrass, green tea, potatoes, algae, and herbs. Chlorophyll and its derivatives are widely used industrially as stable, non-toxic, physiologically harmless colorants for dairy products, edible oils, soups, chewing gums, sugar confections, drinks, cosmetics, and toiletries [1]; they are also used for wound healing, hay fever, and many other conditions, but there is insubstantial scientific evidence to support these uses. Furthermore, the antimutagenic and anticarcinogenic activities of chlorophyll and its derivatives have been proposed [2, 3]. Owing to their anti-oxidant, anti-atherogenic, anti-inflammatory, and detoxification properties, chlorophyll and its derivatives are used in medicines and food supplements [2, 3]. Seaweed and *Spinacia oleracea* have high chlorophyll concentrations. Seaweeds and algae are simple organisms containing chlorophyll [2, 3], consisting of one cell or cells clustered in colonies, often collaborating as simple tissues converting solar energy through photosynthesis to chemical energy [4]. In addition to chlorophyll, several bioactive substances can be utilized for commercial purposes. Marine algae have special biochemical properties that are absent in higher plants [5]. They have a wide variety of shapes; from individual to multi-cellular macroalgae [6]. Generally, marine algae are divided into three classes of pigmentation: Phaeophyceae (Brown), Chlorophyceae (Green), and Rhodophyceae (Red) [7]. A wide variety of bioactive secondary metabolites have been identified, including alkaloids, polyketides, cyclic peptides, polysaccharides, sterols, lipids, and glycerol, that are produced by marine algae as antimicrobial, cytotoxic, and bioactive substances [8].

Microalgae are essential fish-feeding ingredients because of their colorful pigment profiles [4]. In addition, they not only provide diverse organisms, especially fish, color, but also function as vitamins that help with the immune system, metabolism, and reproductive capacity [9]. Algae are nutritionally more effective owing to their high protein, carbohydrate, and vitamin contents, in addition to their pigment abundance [4]. Algal species are used in fish farming as a source of pigments, such as carotenoids, astaxanthin, and lutein, particularly in colored fish [10]. In a previous study, *Nostoc ellipsosporum* and *Navicula minima* algae were analyzed as nutrient sources for the goldfish *Carassius auratus* [11].

Ornamental fish can live in artificial environments, and this adds aesthetic value, which is why their rearing is a common and established practice [9]. Fish quality and price are determined by dyes, which are responsible for a variety of colors [12]. Various plant species and algae, which are considered dye-making agents, are added to the fish diet because fish cannot make all their dyes by themselves [9]. Several coloring agents, which add color to the muscles and skin of the fish, are added to the aqua. Pigmentation is a fundamental criterion for fish as it is the basis for commercial acceptability [12]. *Carassius auratus* has a vibrant color, which is why it is a common ornamental fish [13]. *Carassius auratus* individuals vary in color from gold to yellow or white, with an elongated body and long dorsal fin [14, 15]. This type of fish can survive between freezing temperatures and 30 °C, but the optimal temperatures for its growth and reproduction are above 15°C [9]. *Carassius auratus* breeding has become widespread because of its resilience, as it does not require much protein in its diet and depends on natural food sources [16].

Spinach (*Spinacia oleracea*) is a vegetable rich in vitamins and minerals [17], as well as antioxidant properties such as vitamins E and C [18, 19]. Anti-obesity and lipid-lowering studies in mammalian models have established the effects of *S. oleracea* on health [17, 20]. Found that *S. oleracea* contains high levels of vitamins, lutein, and carotenoids. These carotenoids are characterized by their antioxidant and reactive chemical element species [21, 17]. Reported that green leafy vegetables contain nutrients and non-essential chemical compounds that facilitate health support. *Spinacia oleracea* is a beneficial food source because of its diverse organic composition.

Chlorophyll must protect its properties from chronic maladies [2, 3]. Previous studies have shown that the effects of chlorophyll on obesity [22], such as the supplementation of thylakoids, may suppress weight gain and body fat [23]. In recent years, a large percentage of the world's population has been overweight; this is often thought of as an increasing epidemic that is related to many chronic diseases like polygenic disease, vessel diseases, and cancer [24]. It had become the priority of medical professionals to develop novel pharmaceutical targets to provide obese patients with a more effective form of treatment [24]. Previous studies have shown that the chlorophyll content of some plants, such as *S. androgynous*, possesses substantial nutrient value because of antioxidant interactions [22]. They exhibit alloxan-promoted antidiabetic effects in diabetic mice [24]. In addition, chlorophyll is effective against cardiovascular disorders in Wistar male rats stimulated with a fatty diet and an anti-dyslipidemia [24].

In this study, chlorophyll was extracted from seaweeds and *S. oleracea*. Its potential as an alternative natural food source for *C. auratus*, as well as an efficacious agent for regulating lipid and cholesterol levels in adult male Wistar Hannover rats, was investigated.

## 2. Material and Methods

### 2.1 Algae collection

As previously described by [25], samples were manually collected from the coastal waters of the Red Sea in Jeddah, Saudi Arabia. Several algal species, including ones that belong to Chlorophyta, Phaeophyceae, and Rhodophyceae, were collected. All samples were taken from rock debris; fresh water was then used to remove surface salt. After cleaning, algae were dried in a dry environment for two days;

samples were then kept in a plastic dark bag in the refrigerator at a temperature of 7 °C for further studies.

### 2.2 Spinach leaves collection

As previously described by [26], fresh *S. oleracea* leaves were bought from a local market in Jeddah, Saudi Arabia. The samples were then washed with fresh water to remove any remaining specks of dust and debris. After washing, spinach leaves were dried in a hot oven at 130 °C for about 7 min. Afterward, the leaves were left to cool for 5 min, tightly sealed in a dark plastic bag, and kept in the refrigerator at 4°C until extraction in the laboratory.

### 2.3 Chlorophyll extraction

Chlorophyll extraction was performed as previously described by [26] with some modifications. After washing and drying the seaweed and *S. oleracea* leaves, each one was ground using an electric blender (CH580, Kenwood) until they became a powder, and then placed in an empty plastic bottle with 95 % ethyl alcohol. Each mixture was shaken using a Digital Orbital Shaker (SHO – 2D) at a speed of 120 rpm for 48 h at room temperature. Following homogenization, the suspension was filtered by using filter paper (Whatman, 32.0 cm) and then concentrated under a rotary evaporator (RE-501) at -1 °C and 50–60 rpm for 3 h to obtain a heavy dark viscous green sample. Each sample was precipitated at room temperature to obtain a condensed chlorophyll extract. The extract concentration was 100%, according to the equation described by [26] in Figure 1.

### 2.4 *C. auratus* bioassay

*Carassius auratus* were obtained from a local market in Makkah city and were bred in two tanks (16 liters); the temperature was set at a range of 21 °C±4 °C and pH 8. Fish were starved for 24 h and weighed before feeding. Three replicates were used for each experiment (three fish per replicate). The treated fish were fed with chlorophyll extract (0.6 mg) twice daily for 10 days. The control group was fed commercial food (Aquav Goldfish Flakes).

### 2.5 Wistar Hannover bioassay

This experiment was prepared according to [27]. Eight male albino rats were purchased from King Abdulaziz University (Jeddah, Saudi Arabia) at four weeks of age (200–250 g of weight), and were kept under controlled conditions (20±4 °C, 50±5% R.H, and 12 h light/12 dark cycle) with free access to food and water. They were randomly split into two groups with five individuals each. The control group was fed normal saline, while the treated group was gavage-supplemented with total *S. oleracea* extract once per day in the morning in a starved state at a dose of 2 mg per 250 g of body weight. The total *S. oleracea* extract was suspended in a normal saline volume of 12 ml when gavage was performed and blended using a shaker. Body weight and food intake were recorded daily before gavage. After 14 days, the rats were dissected after 12 h of fasting to prevent cervical dislocation. Blood was collected from the eyes and centrifuged at 3000 rpm for 10 min. The serum was preserved at -80 °C for further analysis.

## 3. Results and Discussion

In this study, chlorophyll was extracted from seaweeds and *S. oleracea* and used as an alternative food source for *C. auratus* and as a lipid and cholesterol regulator in Wistar Hannover rats. A short-term laboratory feeding trial was conducted to assess the relevance of an algae-based value-added feed for *C.*

*auratus*. In comparison to the control group, the mean analysis revealed a substantial increase in body weight in all treated fish compared to the control (18.7, 16.7, 19.8, 19.2, 19.6, and 16.95) as in Table (1). In addition, the algal-induced goldfish color was more vivid. In a similar study, [28] investigated the effect of various carotenoid exporters/concentrations and temperatures on the skin pigmentation of *C. auratus* and found an overall color improvement in goldfish that have been nourished with a high concentration of *Chlorella vulgaris*. In another study by [28], *Ulva reticulata* improved the growth performance and coloration of *C. auratus*.

Also in this study, *S. oleracea* was collected, subjected to alcoholic extraction, and fed to male rats for 14 d. As shown in Table (2), the body weight of the treated rats was significantly higher than that of the control rats. In addition, based on our results in Table (3), there is substantial evidence that spinach increased cholesterol levels in treated rats compared with the control group.

**Table 1:** The mean and standard deviation of treated and control feeding *C. auratus* wight on extracted seaweeds.

Treated fishes	Mean	Standard deviation
F1	18.7	1.85
F2	16.7	1.22
F3	19.8	1.71
F4	19.2	1.84
F5	19.6	1.50
F6	16.95	1.66
Control	20.9	0.20

**Table 2:** Wights of treated adult male Westar rats with *S. oleracea* for 14 days

Treated rats	Wight of treated rats					
	Day 1	Day 4	Day 7	Day 10	Day 14	Average
R1	245	246	243	250	250	246.8
R2	261	260	258	255	252	257.2
R3	263	260	259	257	253	258.4
R4	241	240	231	242	242	239.2
R5	254	252	252	259	261	255.6
Control 1	269	273	275	278	280	275
Control 2	270	277	272	280	289	277.6
Control 3	273	279	281	284	289	281.2

**Table 3:** The effect of *S. oleracea* on the lipid profile of adult male Westar rats.

Groups	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
1	123	75	45	78
2	134	70	40	94
3	129	71	42	87
4	120	76	44	76
5	130	72	42	88
Control	138	91	50	88

Additionally, the levels of triglyceride (TG) and high-density lipoprotein (HDL), the latter of which is known as the “good” cholesterol, were significantly higher in the treated rats compared to the control group. No significant differences were found in the levels of low-density lipoprotein, also known as the “bad” cholesterol, between the groups. In a similar study [29], found that the antioxidant activity of spinach may be effective in overcoming high fat and cholesterol levels [29]. Reported that spinach extract has protective and therapeutic effects in the prevention and treatment of

nonalcoholic fatty liver disease. Surprisingly, [30] reported that the serum cholesterol levels in rats fed with spinach leaf isolate were considerably lower than those in the control group. There are several possible explanations for these results, one of which is that the dose and treatment period differed between the experiments. The method of spinach extraction is also a critical factor to consider. Overall, this study highlights the need for further investigation of the different health benefits of spinach.

#### 4. Conclusions

Chlorophyll extracts from seaweeds and *S. oleracea* administered to *C. auratus* and Wistar Hannover rats showed significant effects. These results indicate that seaweeds could be a better alternative for feeding *C. auratus* than commercial food. *Spinacia oleracea* significantly increased the cholesterol, TG, and HDL levels of Wistar Hannover rats, which can be an effective method to increase lipid and cholesterol levels in people with vascular conditions.

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