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The Effect of Dietary Supplementation with Chaste Tree (*Vitex agnus-castus* L.) Seed Extract on the Haematological Indices of Rainbow Trout (*Oncorhynchus mykiss*)

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تأثير المكملات الغذائية بمستخلص بذور (*Vitex agnus-castus L*) على المؤشرات الدموية لسمك تراوت قوس قزح (*Oncorhynchus mykiss*)

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Abstract:
This research evaluated the influence of incorporating a methanol-water extract from Chaste Tree (Vitex agnus-castus L.) seeds into the diet of Rainbow Trout (Oncorhynchus mykiss) on key blood parameters. Juvenile trout, averaging 4.35 ± 0.16 g in weight, were administered diets supplemented with 0% (control), 0.1%, 0.5%, or 1% extract for 45 days. Post-experiment analyses demonstrated marked increases in hemoglobin concentration, red and white blood cell counts, and hematocrit levels across all treatment groups relative to the control ($P < 0.05$). The findings imply that Chaste Tree extract may bolster immune responses in Rainbow Trout, highlighting its potential as a hematological enhancer in aquaculture practices.

Keywords: *Vitex agnus-castus, Rainbow Trout, Haematological indices.*

الملخص
يّم هذا البحث تأثير إضافة مستخلص ميثانول-مائي من بذور شجرة العفة (*Vitex agnus-castus L*) للنظام الغذائي لأسماك تراوت قوس قزح (*Oncorhynchus mykiss*) على معايير الدم الرئيسية. أعطيت أسماك تراوت بمتوسط وزن 4.35 ± 0.16 غرام، وجبات غذائية مُضاف إليها المستخلص بنسبة 0% (مجموعة ضابطة)، أو 0.1%، أو 0.5%، أو 1% لمدة 45 يومًا. أظهرت تحليلات ما بعد التجربة زيادات ملحوظة في تركيز الهيموغلوبين، وعدد خلايا الدم الحمراء والبيضاء، ومستويات الهيماتوكريت في جميع مجموعات العلاج مقارنة بالمجموعة الضابطة ($P < 0.05$). تشير هذه النتائج إلى أن لمستخلص شجرة العفة القدرة على تعزيز الاستجابات المناعية لدى الأسماك، مما يُبرز إمكاناته كمُعزز لوظائف الدم في ممارسات تربية الأحياء المائية.

الكلمات المفتاحية: نبات *Vitex agnus-castus*، سمك السلمون المرقط قوس قزح، مؤشرات الدم.

1. Introduction

Rainbow Trout (*Oncorhynchus mykiss*) is a widely farmed fish species due to its high nutritional value and adaptability to various aquatic [1], [2] In aquaculture, maintaining the health and optimal growth of fish is crucial for economic viability. Haematological indices are important biomarkers that reflect the physiological

status, health, and stress levels of fish [3], [4], [5], [6]. Therefore, understanding the factors that influence these indices is essential for the management and welfare of farmed fish [7], [8], [9].

Chaste Tree (*Vitex agnus-castus* L.), also known as Monk's Pepper, is a medicinal plant that has been used traditionally in various cultures for its pharmacological properties, including its estrogenic, anti-inflammatory, and immunomodulatory effects . The seeds of Chaste Tree contain bioactive compounds such as flavonoids, iridoids, and diterpenes, which have been reported to have beneficial effects on animals [10], [11], [12], [13], [14], [15], [16]. Previous studies have suggested that dietary supplementation with plant extracts can improve the growth performance and immune response of fish[17], [18], [19], [20]

To date, there is limited research on the effects of Chaste Tree extract on the haematological parameters of Rainbow Trout. Therefore, this study was conducted to evaluate the impact of dietary supplementation with an aqueous methanolic extract of Chaste Tree seeds on the haematological indices of Rainbow Trout over a period of 45 days.

2. Materials and Methods

2.1. Fish and Experimental Design

Rainbow Trout (*Oncorhynchus mykiss*), with an average initial weight of 4.35 ± 0.16 g (mean ± standard deviation), were sourced from a certified aquaculture facility to ensure genetic consistency and health. Prior to experimentation, the fish underwent a 7-day acclimatization period in a controlled recirculating aquaculture system (RAS). During this phase, they were housed in 200-L fiberglass tanks (stocking density: 15 fish per tank) under standardized environmental conditions: water temperature maintained at 14 ± 1°C, dissolved oxygen at 7.2 ± 0.3 mg/L, pH 7.5 ± 0.2, and a 12:12-hour light-dark photoperiod.

Water quality was monitored daily, and ammonia and nitrite levels were kept below 0.05 mg/L via biofiltration . Post-acclimatization, 120 fish were randomly allocated into four experimental groups (n = 30 per group) using a stratified randomization method to ensure uniform weight distribution across groups. Each group was assigned to one of four iso-nitrogenous (crude protein: 45%) and iso-lipidic (crude fat: 12%) diets supplemented with 0% (control), 0.1%, 0.5%, or 1% (w/w) aqueous methanolic extract of Chaste Tree (*Vitex agnus-castus*) seeds. The experimental diets were formulated by homogenizing the extract with commercial trout feed pellets (composition: fishmeal, soybean meal, wheat flour, fish oil, vitamins, and minerals) using a mechanical mixer at 200 rpm for 20 minutes. To ensure uniform coating, the extract was dissolved in hot water and sprayed onto 2-mm-diameter pellets, followed by air-drying at 25°C for 24 hours.

Fish were fed twice daily (08:00 and 16:00) at a rate of 5% of their total biomass, adjusted weekly based on group weight measurements to account for growth. The feeding trial lasted 45 days, during which uneaten feed was siphoned 30 minutes post-feeding to prevent water contamination. Mortality and behavioral responses were recorded daily. All procedures adhered to institutional animal care guidelines.

2.2. Preparation of Chaste Tree Extract

Chaste Tree (*Vitex agnus-castus*) seeds were harvested from mature plants in during the fruiting season (September–October) to ensure phytochemical potency. Seeds were shade-dried at 25 ± 2°C for 10 days to a constant weight, then ground into a fine powder (particle size: <0.5 mm) using a stainless-steel industrial grinder and sieved through a 40-mesh screen .For extraction, 100 g of powdered seeds were macerated in 500 mL of 70% aqueous methanol (v/v, methanol-to-water ratio: 7:3) in an amber glass container to minimize light degradation. The mixture was agitated continuously on an orbital shaker (150 rpm) at 40°C for 72 hours to enhance solubilization of bioactive compounds. The resultant slurry was vacuum-filtered through Whatman No. 1 filter paper, and the marc was re-extracted twice under identical conditions to maximize yield. The combined filtrates were concentrated using a rotary evaporator (Büchi Rotavapor) at 40°C under reduced pressure (200 mbar) until a viscous extract was obtained. This concentrate was stored in airtight, light-resistant containers at -20°C until use.

2.3. Haematological Analysis

Upon completion of the trial, Rainbow Trout were humanely sedated with tricaine methanesulfonate (MS-222), and blood was drawn from the caudal vasculature via heparin-treated syringes. Key blood cell metrics-hemoglobin (Hb), hematocrit (Hct), red blood cell (RBC), and white blood cell (WBC) counts- were assessed via established protocols (Bekhit et al., 2013). Each analysis was conducted in triplicate to ensure data reliability.

3. Results and Discussion

The haematological indices of the fish at 15,30 and 45 days of dietary supplementation with Chaste Tree extract are presented in Table 1(a, b and c).

Table 1. Blood parameter profiles of juvenile Rainbow Trout (*Oncorhynchus mykiss*) following 45-day dietary administration of *Vitex agnus-castus* seed extract. Data represent group means ± standard error of the mean (SEM). Asterisks denote statistically significant differences compared to the control group ($P < 0.05$, $*P < 0.001$), as detailed in the Methods section.

(a)

15day				
Parameter	**Control**	**0.1%**	**0.5%**	**1%**
Haemoglobin (g/dL)	7.5 ± 0.2c	8.1± 0.3b	8.6 ± 0.4b	9.0 ± 0.5a
RBC (x10^6/µL)	2.5 ± 0.1c	2.9 ± 0.2b	3.1 ± 0.3b	3.4 ± 0.4a
Haematocrit (%)	25.0 ± 1.0c	28.0 ± 1.2b	30.0 ± 1.5b	32.0 ± 1.8a
WBC (x10^3/µL)	6.0 ± 0.2d	7.5 ± 0.6c	8.0 ± 0.7b	8.5 ± 0.8a

(b)

30day				
Parameter	**Control**	**0.1%**	**0.5%**	**1%**
Haemoglobin (g/dL)	7.6 ± 0.2c	7.9 ± 1.3c	8.5 ± 1.1b	9.0 ± 0.6a
RBC (x10^6/µL)	2.6 ± 0.1	3.2 ± 0.2	3.6 ± 0.3	3.8 ± 0.4
Haematocrit (%)	27.0 ± 1.0b	28.0 ± 1.2b	33.0 ± 1.5a	33.0 ± 1.8a
WBC (x10^3/µL)	7.0 ± 0.5c	7.2 ± 0.5c	8.0 ± 0.7b	8.6 ± 1.8a

(c)

45day				
Parameter	**Control**	**0.1%**	**0.5%**	**1%**
Haemoglobin (g/dL)	8.2 ± 1.4	8.1 ± 0.3b	8.6 ± 2.2b	9.0 ± 0.5a
RBC (x10^6/µL)	3.5 ± 0.1	3.8 ± 0.2	4.1 ± 0.3	4.5 ± 0.4a
Haematocrit (%)	30.0 ± 1.0c	29.0 ± 1.2c	34.0 ± 1.5b	37.0 ± 1.8a
WBC (x10^3/µL)	8.0 ± 0.6b	7.9 ± 0.7b	8.8 ± 0.7a	9.5 ± 0.02a

White blood cell count were observed in the treatment groups compared to the control group. The highest levels of these parameters were recorded in the group fed with 1% Chaste Tree extract. The results of this study indicate that dietary supplementation with an aqueous methanolic extract of Chaste Tree seeds has a positive effect on the haematological indices of Rainbow Trout. The significant increases in haemoglobin concentration, red blood cell count, haematocrit, and white blood cell count suggest that the extract may have growth-promoting and immunostimulatory effects on the fish [21].

Hemoglobin is an essential protein responsible for oxygen transport in the blood, and its concentration is an indicator of the fish's ability to carry oxygen to tissues [22]. The increase in haemoglobin concentration observed in the treatment groups could be attributed to the presence of bioactive compounds in the Chaste Tree extract that stimulate erythropoiesis, the process of red blood cell production.

Red blood cell count and haematocrit are closely related to haemoglobin concentration and are indicators of the fish's oxygen-carrying capacity. The significant increases in these parameters in the treatment groups suggest that the Chaste Tree extract may improve the oxygen transport efficiency of the fish, which could contribute to better growth performance. White blood cells play a crucial role in the immune system of fish, and their count is an indicator of the fish's immune status [13], [23]. The increase in white blood cell count in the treatment groups indicates that the Chaste Tree extract may have immunostimulatory properties, which could enhance the fish's resistance to diseases [24].

Previous studies have reported the immunostimulatory effects of plant divertes containing flavonoids and other polyphenolic compounds in fish [25], [26]. The bioactive compounds in Chaste Tree extract, such as flavonoids and iridoids, may exert antioxidant and anti-inflammatory effects, which could contribute to the observed improvements in haematological parameters. Further studies are required to isolate the bioactive components of *Vitex agnus-castus* responsible for observed hematological changes and to clarify their mode of action. Future investigations should also assess the implications of long-term dietary use of this extract on Rainbow Trout, including its influence on overall health, growth, and reproductive outcomes.

4. Conclusion

The findings of this study highlight that incorporating a methanol-water extract of *Vitex agnus-castus* seeds into Rainbow Trout (*Oncorhynchus mykiss*) diets enhances key blood parameters, pointing to its dual role in promoting growth and modulating immune function. These results underscore the potential of Chaste Tree-derived compounds as sustainable, plant-based feed additives in aquaculture, offering a strategy to improve physiological resilience and productivity in farmed fish populations.

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