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Effect of different levels of nutmeg on some blood parameters of common carp *Cyprinus carpio* L.

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Abstract

This study was aimed to the effect of different levels of nutmeg on some blood parameters of common carp, it was conducted at special cages in mud pond, Agricultural Research and Experiment Station, Animal Production Department, Agriculture College, Al-Muthanna University at Samawa city. A total of 100 common carp fish, *Cyprinus carpio* L. were brought from a private fish rearing lake in Al-Qadisiyah Governorate, Al-Mihnawiya District, with an average weight of 94 g. 80 fish were selected and distributed randomly and evenly to the experimental cages, put in each cage 5 fish for the purpose of acclimatization, the fish persisted during the acclimatization period, which amounted to 10 days, were starved for one day and then fed with 1% of the weight of the live mass in each tank at the rate of two meals per day. The four treatments were T1 control, T2 0.005%, T3 0.01% and T4 0.015% nutmeg. The results showed that there were no significant differences on the blood traits (hemoglobin concentration, Packed Cell Volume, red and white blood cells), also, there were no significant differences in the biochemical blood traits (total protein, albumin, globulin and glucose).

Keywords: nutmeg, common carp *Cyprinus Carpio* L., blood traits.

Introduction

Despite the role and importance of fish farming as a food source for most of the world's population, however, it faces many challenges that hinder its sustainability and development. Diseases affecting fish are one of the main factors, that limit aquaculture development and culture (Stentiford *et al*., 2017). The expansion of world trade and increased demand for fish, led to the expansion of fish farming according to intensive

systems, that facilitated the spread and development of the deadliest pathogens and the spread of infectious diseases, in addition to exposure to some severe weather phenomena such as drought, storms and high temperatures, which negatively affects the quality of water, this exposes animals to stress and deteriorates their immune system (Pulkkinen *et al*., 2010; Abdel-Tawwab *et al*., 2019; Reverter *et al*., 2020). Despite the efforts and efforts made by specialists in the field of disease control and management, however, the losses caused by disease outbreaks in fish farms around the world

Especially in developing countries, it is estimated at 9.5 billion US dollar annually (Shinn *et al*., 2015). In order to reduce the economic losses due to the outbreak of diseases, threatening the livelihoods of fish farmers, they resort to the use of antibiotics and veterinary chemical drugs,

Frequent use of these substances has negative side effects, whether health or economic, as it contributes to weakening the immune system of fish in addition to the emergence of antibiotic-resistant bacteria and pollution of the aquatic environment, in addition, these drugs and vaccines are very expensive (Cabello *et al*., 2016; Yang *et al*., 2017).

Several researchers have reported anti-inflammatory activity of nutmeg as well as its oil (Mueller *et al*., 2010). Similar to non-steroidal anti-inflammatory drugs, the pharmacological activities are also shown by nutmeg oil (Olajide *et al*., 2000). But the anti-inflammatory activity is only shown by petroleum ether extracts, the alcoholic extract of nutmeg activates an enzyme, it is AMP-activated protein kinase for the treatment of metabolic syndrome including type 2 diabetes and obesity, seven compounds such as tetrahydrofuroguaiacin B, 2,5-bis-aryl-3,4-dimethyltetrahydrofuranlignans,

fragransin C1, saucernetindiol, nectandrin B, verrucosin, galbacin and nectandrin A were isolated from this extract as active components, as well as the production of some isolated compounds potent stimulation of AMPK in differentiated C2C12 cells, at a concentration of 5 µM.

Nutmeg and its active ingredients are not only used to treat type 2 diabetes and obesity but also to reduce metabolic disorders (Nguyen *et al*., 2010).

The current study aims to use nutmeg, Myristica fragrans, in feeding the common carp, *C. carpio* L., and the statement of its effect on blood parameters.

Material and methods

Experience fish

100 common carp *Cyprinus carpio* L. fish, were brought from a private fish rearing lake in Al-Qadisiyah Governorate, Al-Mahanawiya District, with an average weight of 94 g, it was conducted at special cages in mud pond, Agricultural Research and Experiment Station, Animal Production Department, Agriculture College, Al-Muthanna University at Samawa city, from 1/10/2020 to 10/12/2021, after the fish arrived at the experiment site, they were placed in plastic basins that were previously prepared as a 3% salt bath for 5 minutes, u Until signs of stress appear on the fish, in order to eliminate external parasites if they are present in the fish, then the fish were transferred and placed in the iron cage inside the earthen pond and close to the experiment cages for a period of 24 hours, during which the dead fish were excluded

Acclimatization periods

On the next day, the fish were taken out of the iron cage and 80 fish were selected from them and distributed randomly and evenly to the experiment cages, as put in each aquarium 5 fish for the purpose of acclimatization. The fish persisted during the acclimatization period, which amounted to 10 days, the fish were starved for one day and then fed with 1% of the weight of the live mass in each tank at the rate of two meals per day.

Feed material used

Nutmeg

Nutmeg was obtained by buying it from local markets for use in the composition of diets, the diet was made in a tubular shape with a diameter of 3 mm, and after it came out of the pressing machine, it was placed in a large rectangular dish and left to air-dry, then, after drying, it was broken into small pieces 5-10 mm long to make it easier for fish to eat, packaged in 5kg special plastic containers, a sample was taken from the bush for analysis and knowing its chemical composition (Table 2).

Table (1) The composition of the diets used in the experiment.

Items	T1 Control	T2 0.005% Nutmeg	T3 0.01% Nutmeg	T4 0.015% Nutmeg

Protein concentrate*	20	20	20	20
Soybean meal**	35	35	35	35
Bran	15	15	15	15
Maize***	15	15	15	15
Barley	10	10	10	10
Wheat flour	3	3	3	3
Oil	1	0.75	0.5	0
Vitamins and minerals******	1	1	1	1
Nutmeg*******	0	0.005	0.01	0.015
Total	100	100	100	100

* Animal protein concentrate (WAFI) of Dutch origin.

** Soybean meal (EAGLE) of Argentine origin.

*** Yellow Maize (EAGLE) of Argentine origin.

**** Each kilogram contains Vitamin A (400 IU), Vitamin D3 (160 IU), Vitamin E (1200 mg), Vitamin B1 (120 mg), Vitamin B2 (280 mg), Vitamin B6 (160 mg), vitamin B12 (1400 mg), calcium (20.08%), phosphorous (4.90%), sodium (5%).* Nutmeg was added to the rations at an amount (5 g, 10 g, 15 g, per 1 kg of ration).

Table (2) Chemical analysis of the substances included in the composition of the experiment's diets.

Items	**Protein (%)**	**Ethre extract (%)**	**Ash (%)**	**Fiber (%)**	**Carbohydrates (%)**
Protein concentrate*	40	5	23.45	2.81	28.74
Soybean meal**	43.8	2.72	7.21	6.9	39.37
Maize**	9.68	5.04	2.09	2.72	80.27
Barley**	11.83	1.53	4.11	7.0	75.81

Wheat bran**	15.72	4.47	5.52	11.8	62.49
Wheat flour**	10.5	1.5	0.44	0.5	76.0

Chemical composition of experiment diets

Humidity	**Protein (6.25×N)**	**Ethre extract**	**Ash**	**Fiber**	**Nitrogen-free extract**	**Energy*** (kcal)**
7.94	**28**	5.66	8.53	4.69	45.18	390.73

Field experience

The experiment lasted for 72 days with acclimatization using four different experimental diets in terms of adding nutmeg percentages, to determine the effect of its use on some productive traits and some physiological, biochemical and immunological characteristics of common carp fish. The four experimental diets were (T1 control, T2 0.005%, T3 0.01%, T4 0.015% nutmeg) with a crude protein percentage of 28% in all diets. The experimental fish were fed 5% of the live weight for the first 40 days of the experiment, it was served 4 meals a day at (7:30 and 10 in the morning, 12:30 in the afternoon and 3 in the evening, then this percentage was reduced to 3% in the last 20 days of the experiment, provided three meals a day at (eight and eleven thirty in the morning and three in the evening), due to the low water temperature and lack of appetite and fish eating the feed provided to them, the amount of feed was adjusted according to the periodic weight of the fish every ten days and to the nearest decimal place.

Results and Discussion

Blood parameters

Red Blood Cell

Table (3) shows that there are no significant differences between the treatments in the experiment, as the T4 recorded the highest mean of $1.65x10^6$ cells/ mm^3, followed by the T2, which recorded $1.32x10^6$ cells/ mm^3, then T3 recorded $1.21x10^6$ cells/ mm^3, and T1 recorded the lowest mean was $1.1x10^6$ cells/ mm^3.

White Blood Cells

There were no significant differences between the treatments in the experiment for the rate of white blood cells, as T3 recorded the highest average of $149.01x10^3$ cells/ mm^3, while T4 recorded $143.39x10^3$ cells/ mm^3, and T1 came in third place with a rate of $138.17x10^3$ cells/ mm^3 The last, T2, recorded an average of $132.65x10^3$ cells/ mm^3.

Hemoglobin (Hb)

There were no significant differences in the concentration of hemoglobin between the treatments, as T4 recorded the highest mean, as it reached 9.8 g/ dl, followed by T3, which amounted to 9.45 g/ 100 ml, and T2 had an arithmetic average of 9.3 g/ dl, while T1 was ranked The latter, as its was 7.7 g/ dl.

Packed Cells Volume (PCV)

There were no significant differences between the treatments in the experiment, as T2 recorded the highest mean of 28.2%, while T1 recorded the lowest mean of 23.15%, then T4, which recorded mean of 26.05%, and then T3, with mean of 25.75%.

Table (3): Blood parameters (mean ± standard error) of common carp fish fed on rations containing nutmeg during the duration of the experiment.

Treatments	**RBC (Cell×10^6)**	**WBC (Cell×10^3)**	**Hb (g/ dl)**	**PCV (%)**
T1	0.06 ±1.1	7.85±138.17	1.1 ± 7.7	2.15 ± 23.15
T2	0.125 ± 1.32	0.81 ±132.65	0.7 ±9.3	2.4 ±28.2
T3	0.035 ±1.21	2.78 ±149.01	0.05±9.45	0.95±26.05
T4	0.025±1.65	4.33±143.39	1±9.8	0.05±25.75
Sig.	N.S	N.S	N.S	N.S

Biochemical blood tests

Total protein concentration

Table (4) shows that there are no significant differences in the percentage of total protein concentration in the blood, as we note that T3 recorded the highest mean, reached 3.015 gm/dL, followed by T4, which recorded 3.00 gm/dL, while T1 and T2 recorded The lowest mean was 2.95 g/dL for both treatments.

Albumin concentration

There were no significant differences on the concentration of albumin protein for the treatments in the experiment, as T3 recorded the highest mean of 1.20 g /dL, followed by T2 with mean of 1.15 g/dL, then T1 with an mean of 1.10 g/dL, and T4 the lowest was 1.05 g/dL.

Globulin concentration

There were no significant differences on the concentration of globulin protein. We find that the highest mean was reached by T4, which recorded 1.95 g/ dL, and T1

came in the second place was 1.85 g/ dL, and then T3 with mean of 1.81 g/ dL, while T2 was The lowest mean was 1.80 g/dL.

Glucose concentration

There were no significant differences between the experimental treatments, as T3 recorded the highest mean 35.05 g/dL, followed by T4 with an mean of 31.2 g/dL, and then T1 with mean of 29.80 g/dL, while the lowest mean was for T2 was 26.15 g/dL.

Table (4): Biochemical parameters (mean ± standard error) of common carp fish fed on rations containing nutmeg during the duration of the experiment.

Treatments	**Total protein (g/ dL)**	**albumin (g/ dL)**	**Globulin (g/ dL)**	**Glucose (g/ dL)**
T1	0.05± 2.95	0±1.10	1.85±0.05	29.80±5.1
T2	0.05± 2.95	1.15±0.05	1.80±0.1	26.15±0.15
T3	0.01±3.015	1.20±0.20	1.81±0.21	35.05±0.05
T4	0 ±3.00	1.05±0.15	1.95±0.15	31.2±3.25
Sig.	N.S	N.S	N.S	N.S

As for the blood parameters, it was noted that there were no significant differences in all the experimental treatments of blood characteristics, whether cellular (red blood cell count, white blood cell count, hemoglobin concentration and the Packed Cell Volume), as well as the biochemical blood characteristics represented by the concentration of total protein, albumin, globulin and glucose. The blood analysis, whether cellular or biochemical, is a vital indicator of the physiological state of the body and can be used to assess the health status of the body (Ismail and Mahboub, 2016).

Rashidian *et al*. (2022) indicated that the addition of nutmeg significantly improved the values of both red and white blood cells at the 2% level of nutmeg, because of its ability to trigger an antioxidant response, which can protect their membranes from hemolysis, which stimulates an increase in the number of red blood cells.

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