

# Investigating the effect of different levels of turmeric powder (*Curcuma langa*) and black pepper (*Piper nigrum*) on body composition of rainbow trout (*Oncorhynchus mykiss*)

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## Abstract

The purpose of this research was to investigate the effect of different levels of turmeric powder (*Curcuma langa*) and black pepper (*Piper nigrum*) on the body chemical composition of rainbow trout (*Oncorhynchus mykiss*). 450 rainbow trout with an average weight of  $24.95 \pm 0.70$  grams in 10 experimental treatments with 3 repetitions in 60 days with levels (zero, 0.25, 0.5 and 1% of diet) two plants were fed from three combined levels (30% turmeric + 70% black pepper, 50% turmeric + 50% black pepper, 70% turmeric + 30% black pepper). At the end of the course, the body composition of the fish was evaluated. The results showed that although the amount of protein, fat and ash in the fish body was not significant, the amount of dry matter and moisture in the fish body was influenced by the independent effect of the percentage of adding herbal supplements. And the lowest percentage of dry matter was observed at the levels of 0.25, 0.5 and 1% compared to the control treatment, and the lowest percentage of moisture was observed in the control group, and other levels showed the highest values compared to the control treatment.

**Keywords:** rainbow trout, turmeric, black pepper, body composition

## Introduction

One of the main challenges in the aquaculture industry, including rainbow trout (*Oncorhynchus mykiss*), is improving food rations to optimize growth and promote fish health. One of the common methods for improving growth indices, survival rate and food efficiency is the use of additives in farmed fish [1]. Herbal additives with active ingredients can be added

to fishmeal as a nutrition and growth stimulant [2] and by having aromatic substances or as digestive stimulants, they can cause food absorption through changing food patterns, secretion of digestive fluids, digestive enzymes, bile and mucus, and finally consuming more food [3].

Turmeric is one of the medicinal plants with antioxidant and anti-inflammatory compounds [4]. Turmeric (*Curcuma longa*) belongs to the Zingiberaceae family, a herbaceous and stable plant with rhizomes that is used as an additive with medicinal properties, and is widely distributed in the warm regions of Asia (e.g. India, Pakistan, Indonesia, southern part of China), Africa and South America [5]. Turmeric powder contains 6.3% protein, 5.1% fat, 3.5% minerals, 69.4% carbohydrates, 13.1% moisture, and 5% curcuminoids containing curcumin and its derivatives [6]. Ferrulic acid and protocatechoic acid are phenolic compounds of turmeric that have antioxidant and antibacterial properties [7,8] and chemical compounds including volatile oil, zincyburn, alpha and beta-turmerine and other substances including Arabinose, fructose, glucose and starch are present in turmeric plant rhizome [9]. The anti-inflammatory, cholesterol-lowering and antioxidizing properties of turmeric, in addition, the anti-cancer, antibacterial and antifungal functions of this plant additive have been researched by [5,6].

Black pepper (*Piper nigrum*) from the Piperaceae family is a perennial woody plant, native to India, Indonesia, Malaysia, South America, and West Indies, but is also widely cultivated in tropical regions [10]. Black pepper fruit consists of 25.5% protein, 5.3% fat, 1.18% minerals, 37.4% carbohydrates and 4.7% moisture content [11,12]. It contains volatile essential oils and alkaloids called chavicin, p-perine, piperidine, and contains linoleic, oleic and palmitic fatty acids. Piperine is an active component of black pepper and its culprit has antioxidant properties that can protect inflamed tissue against peroxidative damage [13]. Antimicrobial, antimutagen, free radical removal, anti-tumor, antidepressant, liver protectant, immune stimulant, antidiarrheal and antispasmodic properties have been reported for black pepper [14].

Several studies have been conducted on the effect of turmeric and black pepper on different species of fish. Among these studies is a study conducted by Matiullah et al. [10]. They showed that Labeo rohita fish with an average weight of 9.9 g for 60 days fed 0.5% black pepper had the highest specific growth index, feed conversion rate, weight gain, protein, fat, ash and fiber. Mohmoud [15] Effects of different levels of turmeric (*Curcuma longa*) and chili pepper (*Capsicum annum* L.) (1, 1.5 and 2%) in growth progression and chemical composition of common carp (*Cyprinus carpio*) and showed that dietary supplementation of fish with medicinal plants significantly reduced fat in fish body composition. Also, the results of studies by Kamali et al. [16]

showed that the effect of chili pepper (*Capsicum annuum*) and ginger (*Zingiber officinale*) on growth indices, survival and composition of the carcass of *Astronotus ocellatus* carcass showed a significant difference between different treatments in protein efficiency ratio.

Curcumin and its derivatives have antimicrobial, antioxidant, anti-inflammatory, appetite-stimulating, immunomodulatory and digestive protective effects on animal health [17]. Despite its beneficial effects, curcumin has certain limitations such as low water solubility (hydrophobic), unstable chemical structure, rapid metabolism and poor absorption in the body. The bioavailability of curcumin varies by species and genus [18]. However, Patil et al. [19] hypothesized that piperine in black pepper could increase curcumin's bioavailability by 20 and activate CYP3A4 in cytochrome P450, uridine diphosphate-glucosyltransferase, p-glycoprotein and the enzyme

responsible for glucuronization. It can also increase the solubility of curcumin [17]. A well-known bioavailability enhancer, piperine is the main active constituent of black pepper [20] and is associated with a 2000% increase in curcumin bioavailability. The combination of curcumin with piperin found in black pepper increases its absorption in the body [18]. Therefore, it appears that poor habitat accessibility is overcome by adding agents such as piperine that increase habitat accessibility.

Therefore, according to the mentioned materials about the characteristics of medicinal plants, the present research was conducted to investigate the body composition of trout-rainbow-rainbow fish fed with a combination of turmeric powder and black pepper. With the aim that this review can contribute to further studies on the mentioned plants and their use in aquaculture.

## Research method

### 1- Preparation and test conditions:

For this purpose, 450 rainbow trout fry were purchased from a reputable fish breeding center located in Mazandaran-Sari province and were adapted to environmental conditions for 14 days. Fish with an average weight of  $24.95 \pm 0.70$  grams were randomly placed in 30 tanks (300 liters with a water volume of 200 liters) with the same conditions of water volume and quantitative and qualitative factors in the form of 10 treatments (15 fish in each treatment and each treatment with three repetitions) were distributed. During the breeding period, the average physicochemical parameters of water, including temperature ( $13.80 \pm 0.78$  °C), pH ( $7.30 \pm 0.2$ ), water hardness ( $600.52 \pm 22.26$  mg/L and the amount of dissolved oxygen ( $7.14 \pm 0.13$  ppm) was measured and recorded.

### 2- Preparing diet and feeding fish:

Turmeric rhizome (C) and black pepper fruit (P) were purchased from a famous attari located in Mazandaran-Sari province and after grinding with commercial food for trout (FFT1) (Faradane Company, Iran) with a combined ratio (30, 50) and 70 percent) at levels (zero, 0.25, 0.5 and 1) percent of the compound diet and after adding distilled water, the plate was prepared [18] and [21]. Fish prepared with diets (abbreviated T1:0, T2:0.25-70C/30P, T3:0.25-50C/50P, T4:0.25-30C/70P, T5:0.5-70C/30P, T6:0.5- 50C/50P, T7:0.5-30C/70P, T8:1-70C/30P, T9:1-50C/50P and T10:1-30C/70P) at the rate of 3% of body weight and twice a day (hours 9 and 16) were fed for 60 days.

### 3-Measurement of chemical compounds of the body:

At the end of the rearing period, two fish were selected from each replication and the fish carcasses were completely ground and mixed with a meat

grinder after removing the head, tail, fins and viscera. The moisture content of the samples was measured by putting them in the oven at 105 degrees Celsius for 12 hours and the samples dried in the oven at 50 degrees Celsius for 24 hours. The dried samples were ground and powdered and the prepared powder was used to determine the approximate composition of the fish carcass. The amount of crude protein was determined using the Kjeldahl method (nitrogen  $\times 6.25$ ), crude fat was determined by the Soxhlet method, and crude ash was determined by burning the sample in an oven at a temperature of 550 degrees Celsius for 24 hours [22].

### 4- Statistical analysis:

This research is in the form of a completely randomized design and factorial experiment, considering two factors including the levels of adding herbal supplements to the diet (zero, 0.25, 0.5, 1% of the diet) and the ratio of the combination of two plants (30% turmeric and 70% black pepper, 50% black pepper and 50% turmeric, 70% turmeric and 30% black pepper) were planned and implemented. Also, a negative control treatment was considered in order to check the effectiveness of each of the treatments containing herbal supplements compared to zero conditions (without herbal supplements). The Shpiro-Wilk test was used to check the normal distribution of the data. The analysis of the data obtained from the factorial test was performed using the two-way ANOVA method at a confidence level of 95%. Then the comparison of the levels of each first factor in each of the levels of the second factor and vice versa was done through Duncan's multi-range test. The results were displayed as mean  $\pm$  standard deviation. Data analysis was done using SPSS 2016 software and the resulting graphs were drawn using Excel 2010 software.

## Findings

### Carcass composition:

The results of the factorial test on the composition of the carcass at the end of the 60th day of sampling showed that the interaction between the two factors and the independent effects of each factor on the composition of the carcass including protein

percentage, fat percentage and ash percentage were not significant ( $P < 0.05$ ) but the independent effect of the first factor in dry matter and moisture was significant ( $P < 0.05$ ) (Table 1).

Table 1- The results of the comparison of carcass composition (percentage of protein, fat and ash) in rainbow trout fry fed with different levels of turmeric and black pepper at the end of the 60th day of feeding (mean  $\pm$  standard deviation)

parameter	combined ratio	percent of the compound diet(%)			
		0	0.25	0.5	1
Protein(%)	70%P+30%C	33.72 $\pm$ 1.14 <sup>A</sup>	32.43 $\pm$ 1.40 <sup>A</sup>	34.88 $\pm$ 0.44 <sup>A</sup>	34.71 $\pm$ 1.49 <sup>A</sup>
	50%P+50%C	33.72 $\pm$ 1.14 <sup>A</sup>	35.00 $\pm$ 1.35 <sup>A</sup>	35.35 $\pm$ 1.53 <sup>A</sup>	39.03 $\pm$ 1.17 <sup>A</sup>
	30%P+70%C	33.72 $\pm$ 1.14 <sup>A</sup>	33.83 $\pm$ 1.49 <sup>A</sup>	34.59 $\pm$ 1.05 <sup>A</sup>	37.98 $\pm$ 1.60 <sup>A</sup>
Fat(%)	70%P+30%C	30.93 $\pm$ 4.63 <sup>A</sup>	32.46 $\pm$ 2.52 <sup>A</sup>	30.95 $\pm$ 1.09 <sup>A</sup>	30.06 $\pm$ 1.99 <sup>A</sup>
	50%P+50%C	30.93 $\pm$ 4.63 <sup>A</sup>	33.66 $\pm$ 1.00 <sup>A</sup>	30.37 $\pm$ 2.07 <sup>A</sup>	30.04 $\pm$ 2.53 <sup>A</sup>
	30%P+70%C	30.93 $\pm$ 4.63 <sup>A</sup>	35.84 $\pm$ 2.96 <sup>A</sup>	30.39 $\pm$ 0.30 <sup>A</sup>	32.14 $\pm$ 1.79 <sup>A</sup>
Ash(%)	70%P+30%C	6.71 $\pm$ 1.96 <sup>A</sup>	5.53 $\pm$ 0.47 <sup>A</sup>	5.42 $\pm$ 0.50 <sup>A</sup>	5.12 $\pm$ 1.17 <sup>A</sup>
	50%P+50%C	6.71 $\pm$ 1.96 <sup>A</sup>	4.84 $\pm$ 0.88 <sup>A</sup>	7.88 $\pm$ 3.91 <sup>A</sup>	5.57 $\pm$ 1.12 <sup>A</sup>
	30%P+70%C	6.71 $\pm$ 1.96 <sup>A</sup>	4.22 $\pm$ 1.11 <sup>A</sup>	8.03 $\pm$ 5.42 <sup>A</sup>	5.13 $\pm$ 1.11 <sup>A</sup>

Same letters in each column and row indicate non-significance at the 0.05 level ( $P > 0.05$ ).

Dry matter percentage and carcass moisture were influenced by the independent effect of the first factor, i.e., the addition of herbal supplement percentage. So that the process of changes in dry matter and moisture was different. While the amount

of dry matter decreased with the increase in the percentage of herbal supplement, in terms of moisture, it had an increasing and constant trend, so that the highest amount was obtained at three levels of 0.25, 0.5 and 1% (Table 2).

Table 2- The results of the comparison of carcass composition (percentage of dry matter and moisture) in rainbow trout fry fed with different levels of turmeric and black pepper at the end of the 60th day of feeding (mean  $\pm$  standard deviation)

parameter	percent of the compound diet(%)			
	0	0.25	0.5	1
Dry matter (%)	31.72 $\pm$ 4.38 <sup>B</sup>	27.56 $\pm$ 0.99 <sup>A</sup>	27.96 $\pm$ 0.58 <sup>A</sup>	27.55 $\pm$ 0.55 <sup>A</sup>
Moisture(%)	68.28 $\pm$ 4.38 <sup>A</sup>	72.44 $\pm$ 0.99 <sup>B</sup>	72.04 $\pm$ 0.58 <sup>B</sup>	72.44 $\pm$ 0.55 <sup>B</sup>

Letters (A-B) in each row indicate a significant difference at the 0.05 level ( $P < 0.05$ )

## Results Discussion

In the last few decades, the use of medicinal plants has attracted a lot of attention worldwide due to its many advantages, including minimal environmental risks, lack of drug resistance, cheapness, stability, and availability. Therefore, among many immune stimulants, immune stimulants of plant origin are preferred [23].

The chemical composition of fish body depends on various factors such as fish species, feed type, age, feeding percentage, feed formulation and water temperature [24]. The results of this research showed the non-significance of the mutual effects of two factors and the independent effects of the factors in the amount of protein, fat and carcass ash ( $P < 0.05$ ). But the percentage of dry matter and carcass moisture was influenced by the independent effect of the first factor. Thus, with the increase in the percentage of herbal supplement, the percentage of dry matter decreased, but the moisture increased and remained

constant. These results are parallel to the results of Ghanbary et al. [25] that the carcass composition of fish fed with TSCNPs had more protein and less fat than other treatments. Also, Firouzabakhsh et al. [26] showed that the amount of crude fat in rainbow trout of the control group was the lowest compared to the groups fed with red pepper extract. In line with the current study, Ebrahimi et al. [27] studied garlic essential oil at 6 levels (without garlic and antibiotic, antibiotic, 50, 100, 150 and 200 mg/kg) and found a significant difference in the amount of protein, fat and ash were not obtained among the treatments compared to the control treatment, which is consistent with the present research. The highest amount of protein in the treatment was 150 mg, which is consistent with the research results of Cho and Lee [28] on different levels of onion powder on flounder and paralectis fish. Wojno et al. [29] showed that no significant difference was observed in total body protein, ash or moisture level in common carp

fed with 0.02% piperine and curcumin. However, the lowest fat concentration was recorded in the casein and gelatin (CG)-based diet group compared to other treatments. On the contrary, the fish of piperine group showed the highest level of fat compared to other groups. But Ullah et al. [30] showed that the amount of protein and body fat in *Labeo rohita* fish fed with 1% of black pepper leaf extract (*Piper nigrum*) increased significantly, while the remaining amounts of approximate composition such as moisture, dry matter and ash had no significant difference. In the results obtained from different studies with different plant derivatives, it can be caused by the difference in compositions, the percentage of effective substances in different plants, fish species and basic diet compositions.

### Conclusions

The results of this research showed that the body moisture and dry matter of rainbow trout were influenced by supplementing the diet with turmeric and black pepper plants, so that the fish fed with the powder of these plants had a significant difference after 60 days of feeding. In the mentioned indicators, they showed that it indicates the effective role of these natural stimulants in fish. Although it did not affect the amount of protein, fat and ash in the fish body. Therefore, it is suggested to conduct more studies on increasing the percentage of turmeric and black pepper in the diet of rainbow trout.

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