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Feeding common carp fish on diets containing different percentages of ginger *(Zingbar officinale)* and its effect on the characteristics of the blood and biochemical parameters

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Abstract: Common carp (*Cyprinus carpio* L.) were manually fed a diet supplemented with ginger powder (*Zingiber officinalis*) at levels (0, 6, 8, and 10) % to observe the effect of ginger powder on blood and biochemical parameters. The research was conducted in the fish laboratory of the Faculty of Animal Production /College of Agriculture and Forestry/University of Mosul. In this study 84 fish were used and distributed over 12 glass tanks with an average weight of 28 ± 1gm. The fish were fed 3 times a day at a rate of 3% of body weight for a period of 56 days. At the end of the experiment, there were significant differences (P<0.05) in the hemoglobin standard in the treatments to which ginger was added compared to the control treatment. PCV values also increased in fish fed on the added diets. Also, albumin values decreased in treatments to which ginger was added compared to the control treatment, and globulin values decreased in treatment 2 (6% ginger). There were also no significant differences in the total protein standard between the control treatment and the treatments to which ginger was added, except for the treatment which had an 8% level of ginger. The level of glucose also decreased significantly in blood of fish fed on ginger compared to the control treatment. while the values of cholesterol and triglycerides varied for the ginger treatments compared to the control treatment. Also the values of Aspartate Transaminase enzyme (AST) decreased in the treatments to which ginger was added compared to the fish fed on the control diet and Alanine Transaminase enzyme (ALT) values also decreased in all treatments that had ginger added to it compared to the control except for the fourth treatment (10% ginger), which was higher than the rest of the treatments. Feeding fish diets containing ginger lowered hemoglobin in the blood.

Keywords: Ginger, Common carp, biochemical parameters, blood standards.

تغذية أسماك الكارب الشائع على علائق تحتوي على نسب مختلفة من الزنجبيل *(Zingbar officinale)* وتأثيره على صفات الدمية والمعايير الكيموحيوية

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المستخلص: تم تغذية أسماك الكارب الشائع (*Cyprinus carpio* L.) على علائق تحتوي على نسب مختلفة من مسحوق الزنجبيل (0 ، 6 ، 8 ، 10)% بهدف دراسة تأثير مسحوق الزنجبيل على الدم والمعايير الكيموحيوية. أجريت الدراسة في مختبرات الأسماك التابعة لقسم الإنتاج الحيواني في كلية الزراعة والغابات/ جامعة الموصل – العراق. أستخدم في هذا البحث 84 من أسماك الكارب الشائع بمعدل وزن 28 ± 1 غم، وزعت الأسماك على 12 حوضاً زجاجياً وتم تغذيتها 3 مرات يومياً وبمعدل 3% من وزن الجسم الحي لمدة 56 يوماً. في نهاية التجربة تم تسجيل فروقات معنوية (P<0.05) في مستوى الهيموكلوبين فيما يخص المعاملات التي احتوت على الزنجبيل بالمقارنة مع معاملة السيطرة، ايضاً كان هنالك ازدياد في مستويات PCV في الأسماك التي تغذت على العلائق المحتوية على الزنجبيل ، بينما لوحظ انخفاض في مستويات الالبومين في المعاملات التي احتوت على الزنجبيل مقارنة بمعاملة السيطرة، تم تسجيل انخفاض في مستوى الكلوبيولين في المعاملة الثانية التي احتوت على(6 % من الزنجبيل)، وتم تسجيل فروقات معنوية في البروتين الكلي في المعاملة التي أحتوت على (8 % من الزنجبيل) مقارنة بمعاملة السيطرة، مستوى الكلوكوز انخفض معنوياً في دم الأسماك التي تغذت على الزنجبيل مقارنة بمعاملة السيطرة، بينما أختلفت قيم الكوليسترول والكليسيريدات الثلاثية في المعاملات التي المحتوية على الزنجبيل مقارنة بمعاملة السيطرة ، ايضاً لوحظ انخفاض في مستويات أنزيم AST في الأسماك التي احتوت علائقها على الزنجبيل مقارنة بمعاملة السيطرة، وانخفض مستوى أنزيم ALT في جميع المعاملات ما عدا المعاملة الرابعة (10 % زنجبيل)التي كانت أعلى من باقي المعاملات. ان تغذية الأسماك على علائق تحتوي على الزنجبيل أدت الى انخفاض في مستوى الهيموكلوبين في الدم.

الكلمات المفتاحية: الزنجبيل، الأسماك، الكارب الشائع، المعايير الكيموحيوية ، الصفات الدمية.

Introduction

Aquaculture is one of the fastest growing sectors as it provides food of high value to humans and plays an important role in improving their health (Mirghaed *et al.*, 2018). Therefore, the production of the aquaculture sector has increased significantly in recent decades (FAO, 2014). Because there is a huge demand for aquaculture products all over the world. Therefore, breeders and farmers have tended to increase fish production through intensive farming (Mirghaed *et al.*, 2018). Intensive fish farming has had adverse effects on the growth performance of fish as well as its health (Yousefi *et al.*, 2016). As the immunity of fish decreases and their resistance to diseases becomes less when cultured at high densities (Fazelan *et al.*, 2020). Therefore, available feed additives are used to improve the growth performance of fish, as some antibiotics and hormones are used as feed additives, which may cause harmful side effects to fish (Mahmoud *et al.*, 2019). Nutritional status acts as a critical factor in the host's defense against pathogens and as such the use of feed additives has been aimed not only at improving growth but also improving health status which has gained them wide interest and acceptance (Shokr and Mohamed.,2019). The frequent application of antibiotics and biocides in aquaculture can result in the development of bacteria that are resistant to antibiotics and the creation of harmful substances, which may present dangers to the ecosystem, as stated by Esiobu *et al.* (2002). Therefore, the World Health Organization has encouraged the use of herbs and medicinal plants to reduce the use of chemicals and replace them by returning to natural sources. The use of natural materials such as plants and medicinal herbs as feed additives is acceptable because they enhance the effectiveness of feed and animal production performance (Lević *et al.,*2008).

Weidner and Sigwart (2000) suggest that Ginger (*Zingiber officinalis*, Roscoe) is generally considered to be a safe herbal medicine. Ginger is known to contain a variety of compounds such as alkaloids, flavonoids, polyphenols, saponin, steroids, tannin, fiber, carbohydrate, vitamins, carotenoids, and minerals (Otunola *et al.*, 2010; Shirin and Prakash, 2010). The natural antioxidants found in ginger, such as gingerols, shogaols, and zingerone, have been identified by Masoud and Mostafa (2014). In addition, essential oils present in ginger have been shown to have potent anti-inflammatory effects, and oleoresin has been studied by Hassanin *et al.* (2014) and Zarate and Yeoman (1996). Ginger is a spice that is reported to have many different benefits for the body, including reducing the risk of heart disease, reducing the risk of blood clots, and reducing the risk of diabetes. It is also known to be effective at improving the symptoms of a wide variety of other conditions, including gastrointestinal problems, cardiovascular problems, and cancer as Studies conducted by Nya and Austin (2009), Apines-Amar *et al.* (2012), and Talpur *et al.* (2013) have demonstrated that supplementing ginger in the diets of humans and animals, including fish, may strengthen host innate immune functions that are essential for protecting against infectious diseases. By doing so, it is believed that ginger may enhance disease resistance in fish.

This study aims to investigate the effect of adding ginger powder on the blood and biochemical characteristics of common carp fish.

Materials and Methods:

Study position:

The research was conducted for eight weeks, from July 27, 2022, to September 27, 2022, at the fish laboratory located in the Faculty of Animal Production at the College of Agriculture and Forestry, University of Mosul.

Fishes experience:

The primary species of fish used for breeding in Iraq is the common carp, (Cyprinus *carpio* L.). To conduct a feeding experiment, 84 common carp fish with an average weight of 28±1 g/fish were placed into 12 glass aquariums that measured 40 x 60 x 40 cm. Each aquarium contained seven fish, and there were three replications per treatment. The fish were given two weeks to acclimate to their new environment before the feeding experiment began.

Breeding water's qualitative characteristics:

To eliminate waste and maintain a safe environment for the fish, de-chlorinated tap water was used in a tank with a capacity of 70 liters, and 20-30 percent of the water was replaced every 24 hours. The following were the averages for certain physical and chemical parameters over the course of the experiment: temperature 24- 26°C, pH 7.7- 7.8 and Dissolved Oxygen 5.5-6.0 mg/l. These parameters are within the appropriate and recommended limits (FAO, 1981).

Experimental diets:

Four experimental diets were prepared. Diets 2, 3 and 4 contained ginger powder *Zingiber officinale*, at 6, 8, 10% the first diet was free of additives (Table 1). The fish were fed at a rate of 3% of their live weight two meals per day.

Statistical analysis:

The statistical analysis of the data was performed using the SPSS software package (version 25, SPSS Inc., USA, 2017) and complete randomized design (CRD). To assess the differences between groups, Duncan's multiple range test (Duncan, 1955) was used. The results were reported as mean±SE, with significance levels set at P≤ 0.05 for significant differences.

Table 1: Dietary ingredient and proximate (% DM) of the experimental diets containing different levels of ginger.

experimental diets / ingredients	Control	Ginger		
		6%	8%	10%
	(1)	(2)	(3)	(4)
animal protein concentrate*	12	12	12	12
Soybean meal	30	30	30	30
ginger powder	-	6	8	10
Wheat bran	19	19	19	19
Yellow corn	16.5	16.5	16.5	16.5
Local barley	20	20	20	20
Binder (bentonite)	0.5	0.5	0.5	0.5
Vita. & Miner. Mix.	0.5	0.5	0.5	0.5
Food salt	1	1	1	1
Lime stone	0.5	0.5	0.5	0.5

chemical analysis						
Dry matter	Moisture	Crude protein	Either extract	Ash	NFE	ME(MG/KG)**
92.41	7.59	25.54	6.34	5.67	54.86	14.35

*** animal protein concentrate WAFI HOLLAND for carp fish (crude protein 37.50 , crude fat 3.50 ,crude fiber 1.14 ,moisture 2.67 ,Ash 37.31 + A mixture of vitamins, minerals, amino acids and antioxidants.**

**** According to Smith's equation (1971): Protein × 18.5 + Fat × 33.5 + NFE × 13.8.**

Hematological and biochemical parameters:

After eight weeks of the study ended, the fish were anesthetized with a spinal cord destruction method (Lucky 1977). Blood samples were drawn from the caudal vein behind the caudal fin, using a 3 mL plastic syringe. The blood samples were divided into two parts, the first section was placed in gel tubes that did not contain an anticoagulant to obtain serum, and the second section of the blood was placed in glass tubes without anticoagulant (heparin). The tubes were left tilted at room temperature for half an hour, and then centrifuged at 3000 rpm for ten minutes. The serum was kept in special tubes at -20 ° C until the completion of the laboratory tests. The second portion of the blood was stored in plastic tubes containing anticoagulants (heparin) for the purpose of subsequent blood tests. The hematological parameters were estimated Included (Hb and PCV). Biochemical parameters included an estimate albumin, globulin, total protein, glucose, cholesterol, triglycerides, ALT and AST.

Results and Discussion:

1- PCV and Hb:

Statistical analysis of blood parameters (as shown in table 2) revealed significant differences (P<0.05) between the third diet (ginger 8%) and the second diet (ginger 6%) in PCV parameter, while there were no significant differences between the third diet and first diet (control) and fourth treatment. As for the hemoglobin in the blood There were significant differences between the third diet and the first, second diets, and there was no significant difference between the third and fourth diets.

Table 2: Effect of Ginger on PCV and Hb of common carp

Criteria / Diets	PCV %	Hb mg\dl
(1) Control	43.6667 ± 0.33 ab	12.2000 ± 0.10 c
(2) Ginger 6%	42.0000 ± 1.00 b	13.5333 ± 0.39 b
(3) Ginger 8%	44.6667 ± 0.66 a	14.5333 ± 0.23 a
(4) Ginger 10%	44.0000 ± 0.57 ab	14.3000 ± 0.17 ab

Means without the same letter were significantly different (P < 0.05).

These results agreed with Abdelmagid *et al.* (2022), where he indicated that the values of PCV and Hb increased in Nile tilapia *Oreochromis niloticus* fish that were fed on diets to which ginger oil was added by four levels compared to the control. It also agreed with Almeida *et al.* (2021) when feeding Neotropical catfish *Pseudoplatystoma reticulatum* on 4 levels of ginger oil, and 0.5% showed a clear increase in Hb level. Chang *et al.* (2021a) indicated an increase in PCV when feeding Nile tilapia juveniles on diets to which different levels of essential oils of ginger were added, as all treatments showed a non-significant superiority with the control diet, and the highest rise was in the diet to which oil was added 1 mL kg diet^{-1}. It was also observed that there was a significant superiority in the PCV standard when feeding common carp (*cyprinus carpio*) fish on diets containing ginger extract, and the highest values were when the extract was added at rates of 2 and 4%, respectively, and hemoglobin in fish fed 2% of ginger was superior (mohammadi *et al,.*2020). Jafarinejad *et al,* (2020) also indicated that there are differences in PCV when feeding common carp juveniles on ginger at a rate of 2 and 5%. Shokr and Mohamed (2019) fed Nile tilapia with diets containing ginger at a rate of 6 and 9 g/kg and found significant differences in PCV. In addition, they found significant differences when fed on diets containing 12 and 15 g / kg, and significant differences were found in standard Hb, while high significant differences were observed when feeding on diets to which ginger was added at a rate of 15 g / kg feed. Gholipourk kanani *et al,* (2014) indicated a significant increase in PCV and Hb values when feeding *Huso huso* fingerlings on diets to which ginger and garlic were added. Chang *et al,* (2021b) also indicated a non-significant increase in PCV

in diets supplemented with ginger oil, as it was highest in tambaqui *Colossoma macropomum* juveniles fish that were fed diets containing (2, 1.5, and 1) mL kg diet-1, respectively, while Hb increased in the blood of the fish which were fed on diets containing (2 and 1.5) mL kg diet-1, respectively. Hb also increased in young Sparidentex hasta when ginger was added by 1% compared to control (Jahanjoo *et al,* 2018). Negm *et al,* (2016) indicated an increase in PCV and Hb when using ginger oil when feeding Nile tilapia fingerlings on diets to which ginger oil was added (0, 1, 2, and 3%) compared to control. The values of PCV and Hb also increased compared to the control when rainbow trout were fed 1% ginger root powder (Haghighi *et al,*2013).

2- Albumin, globulin total protein:

The results obtained from (Table 3) indicated The blood's albumin concentration showed notable distinctions with a statistical significance of (P< 0.05), in which the fish of the first treatment (control) excelled when compared to the other treatments, which had ginger added to their diets. The globulin concentration decreased significantly for the treatments to which ginger was added compared to the control treatment, except for the second treatment, where there was no significant difference between it and the control.

As for the concentration of total protein, it was the highest in fish fed on the diet of the second treatment (ginger 6%), but there were no significant differences between it and the first and fourth treatments as the lowest concentration of total protein for fish was fed on the third diet. The current results also showed that the albumin/globulin ratio in the third and second treatments was significantly superior to the first and fourth treatments.

Table 3: Effect of Ginger on albumin, Globulin, total protein and Globulin/Albumin glo\alb of common carp.

Criteria / Diets	Albumin mg\ dl	Globulin mg\ dl	Total protein mg\ dl	Globulin/Albumin Glo/Alb
(1) Control	0.8467 ± 0.02 a	0.06 ± 2.9867 a	0.52 ± 3.8333 ab	3.4267± 0.16 b
(2) Ginger 6%	0.00 ± 0.7067 b	0.08± 3.2367 a	4.0333 ± 0.29 a	4.6433 ± 0.32 a
(3) Ginger 8%	0.00 ± 0.7133 b	0.13 ± 2.4533 b	0.03 ± 2.9333 b	5.1367± 0.54 a
(4) Ginger 10%	0.00 ± 0.7467 b	0.10± 2.4200 b	3.2000 ± 0.10 ab	3.2733 ± 0.35 b

Means without the same letter were significantly different (P < 0.05).

Serum biochemical measurements are among the most important aquaculture parameters that reflect fish performance and health (Fazio *et al,* 2013; Fazio, 2019). Abdelmagid *et al.* (2022) indicated. That feeding tilapia fish on 4 different levels of ginger oil led to a significant superiority in total protein and albumin. The values of total protein and albumin increased when common carp were fed ginger extract by 0.2 and 0.4%, respectively, and Globulin values increased when fish were fed 0.2% of ginger extract

(Mohammadi *et al*,2020). Shokr and Mohamed (2019) noted that there were significant differences in the level of total protein when feeding Nile tilapia fish on diets containing ginger by 6 and 9 g / kg feed, respectively, and there were high significant differences in the level of total protein by 12 and 15 g / kg feed, respectively. Jahanjoo *et al.* (2018) also indicated an increase in total protein and albumin values compared to control when using 1% ginger oil in *Sparidentex hasta* Fry. Negm *et al.*(2016) indicated a significant increase in total protein and albumin values when Nile tilapia were fed ginger container diets compared to control. The use of ginger and garlic in feeding *Huso huso* fingerlings led to significant differences in the values of albumin, Globulin and total protein compared with the control (Gholipourk kanani *et al*, 2014).

3- Glucose, cholesterol and triglycerides:

The results shown in (Table 4) indicated that there were significant differences (P< 0.05) in the level of blood glucose of fish fed on diets containing ginger. Where the level of glucose increased for all treatments to which ginger was added, and it was the highest for the third diet. The cholesterol level also increased significantly in fish fed on the second and fourth diets compared to the control diet. The cholesterol level remarkably decreased in the third diet compared to the control. In addition, triglycerides levels significantly decreased for the second and third treatments compared to the control treatment but, it was significant higher in the fourth treatment compared to the control.

Table 4: Effect of Ginger on glucose, cholesterol and triglycerides of common carp.

Criteria / Diets	Glucose mg\ dl	Cholesterol mg\ dl	Triglycerides mg\ dl
(1) Control	96.1433± 0.61 d	117.3900± 0.79 b	115.6900 ± 0.77 b
(2) Ginger 6%	131.0000 ± 0.57 b	129.0000 ± 0.27 a	84.5600 ± 0.51 d
(3) Ginger 8%	149.3333± 0.66 a	104.4900 ± 1.20 c	84.5600± 1.12 c
(4) Ginger 10%	112.3333 ± 0.66 c	129.0000± 0.51 a	143.4500 ± 1.07 a

Means without the same letter were significantly different (P < 0.05).

According to Wendelaar Bonga (1997), Abdel-Tawwab (2012), and Ghelichpour *et al.* (2018), cortisol and glucose levels are commonly used indicators of stress in fish, as they tend to increase during stress and lead to higher energy consumption. There were significant differences in the level of blood glucose when feeding Nile tilapia fish on diets containing ginger at the level of 6 and 9 g / kg feed, and high significant differences at 12 and 15 g / kg. It was also noted that there were high significant differences in the cholesterol level when feeding the fish on ginger at the level 6 g/kg and very high when feeding fish at levels of 9, 12 and 15 g/kg. There were also significant differences in the level of triglycerides when feeding Nile tilapia fish on diets containing ginger at levels of 6 and 9 g/kg, and very high differences when feeding on diets containing 12 and 15 g/kg, respectively (Shokr and Mohamed,

2019). Fazelan *et al.* (2020) also indicated that feeding common carp fish divided into four groups at high densities on diets to which ginger was added at an amount of 5 and 10 g / kg had an effect on reducing stress.

4- ALT and AST: (Alanine transaminase & Aspartate transaminase).

According to the statistical analysis presented in Table 5, it was found that there were notable variations with a significance level of ($P<0.05$) in the levels of liver enzymes. Where the level of ALT enzyme increased significantly in the fish fed on the fourth diet (ginger 10%) compared to the control diet. While the concentration of ALT enzyme decreased significantly in the fish of the second and third treatments, compared to the control diet. As for the AST enzyme, the level of the enzyme decreased in all fish that were fed on diets containing ginger compared to the control.

Table 5: Effect of Ginger on ALT and AST of common carp.

Criteria / Diets	ALT (IU/L)	AST (IU/L)
(1) Control	165.00 ± 1.73 b	311.33 ± 3.71 a
(2) Ginger 6%	158.33 ± 2.02 c	284.00 ± 1.52 b
(3) Ginger 8%	112.66 ± 1.20 d	216.00 ± 2.08 d
(4) Ginger 10%	173.66 ± 2.33 a	238.66 ± 2.96 c

Means without the same letter were significantly different ($P < 0.05$).

These results agreed with Shokr and Mohamed 2019, where it was noted that there were significant differences in the level of ALT when feeding Nile tilapia fish on diets containing ginger at an amount of 6 and 9 g/kg. There were also significant differences in the level of AST when feeding Nile tilapia fish on diets containing ginger at an amount of 6 g/kg. There were also high significant differences in the content of ALT when feeding Nile tilapia on diets containing ginger at an amount of 12 and 15 g/kg, and there were high significant differences in AST content when feeding Nile tilapia on diets containing ginger at an amount of 9, 12 and 15 g/kg, respectively. While Oh *et al.* (2022) indicated that there were no significant differences in liver enzyme ALT and AST in black rockfish (*Sebastes schlegelii*) fed on diets to which ginger was added with the control diet.

Conclusion

When fish were fed diets that included ginger, it led to a reduction in hemoglobin levels in their blood. In addition, the presence of 8% ginger in their diet resulted in decreased levels of liver enzymes (ALT, AST), albumin, globulin, total protein, cholesterol, and triglycerides in their blood. Conversely, the glucose level in their blood increased when fed a diet containing 8% ginger.

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