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ORIGINAL ARTICLE

Effect of replacing dietary fish meal with silkworm (*Bombyx mori*) pupae on hematological parameters of rainbow trout *Oncorhynchus mykiss*

Matin Shakoori · Hamed Gholipour · Samira Naseri

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Abstract In this study, the effects of substituting fish meal with different levels of silkworm pupae on hematological parameters of rainbow trout *Oncorhynchus* were investigated over the course of a 60-day experiment. For this purpose, four treatment groups including T_1: with 100 % fish meal (FM), T_2: 5 % silkworm pupae (SP) + 95 % FM, T_3: 10 % SP + 95 % FM, and T_4: 15 % SP + 95 % FM and one control group were considered for the experiment. Each treatment group was divided into three replicates of 30 fish per replicate. According to the results, the values of red blood cells (RBCs) and hemoglobin (Hb) were decreased significantly with increasing of silkworm pupae percent in diet. In this regard, the lowest values of RBCs and Hb were observed in T_4. There were no significant differences between experimental treatments in terms of hematocrit values. The MCH, MCV, and white blood cell values increased with increasing of silkworm pupae percent in diet. Also, the MCHC values did not show significant differences between experimental treatments. In conclusion, our results showed that incorporation of silkworm pupae in diet can affect health status of rainbow trout.

Keywords Fish meal · Silkworm pupae · Hematological parameters · Rainbow trout

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Introduction

Fish meal is used widely as integral part of commercial diets to provide the protein requirements of cultured fish. Nevertheless, use of fish meal is very expensive and increases the costs of rainbow trout aquaculture. During the past decades, numerous inexpensive protein resources were experienced as replacement for fish meal. The protein resources such as soybean (Hardy 1982; Pongmaneerat and Watanabe 1993; Oliva-Teles et al. 1994; Kaushik et al. 1995; Webster et al. 1992; El-Dahhar and El-Shazly 1993), maize gluten meal (Wu et al. 1995), lupins (Fontainhas-Fernandes et al. 1999), rapeseed (Davies et al. 1990), cottonseed meal (Rinchard et al. 2002), corn gluten (Moyano et al. 1992; Robaina et al. 1995; Jahanbakhshi et al. 2012), and canola meal (Hardy and Sullivan 1983; Lim and Klesius 1998; Abbas et al. 2008; Thiessen et al. 2004). In addition to these sources, the silkworm pupae have been found to be one of the best substitutes to fish meal in the diets of some cultured fish species especially carps (Nandeesha et al. 1990, 1999, 2000; Habib et al. 1994; Mahata et al. 1994; Rangacharyulu et al. 2003). Generally, a protein resource is useful that provides fish requirements and also guarantees fish health. Several studies have reported that the dietary fish meal could be replaced with various levels of silkworm pupae without adverse effects on growth and survival parameters. The hematological characteristics of fishes are an integral part of evaluating their health status (De Pedro et al. 2005; Martins et al. 2008). To our knowledge, there was no information about the effects of silkworm pupae as a protein source on hematological parameters of rainbow trout. In this study, we investigate the effects of substituting fish meal with different levels of silkworm pupae on hematological parameters of rainbow trout.

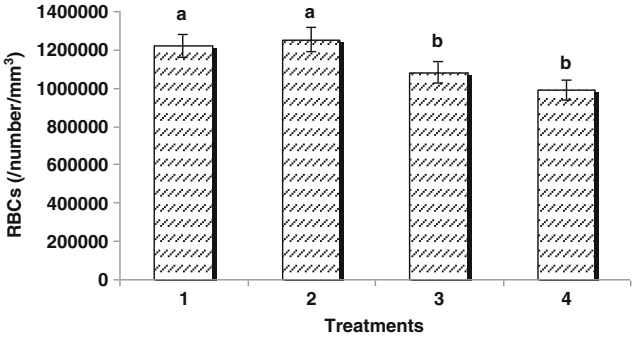

Fig. 1 Comparison of RBC count between experimental fish groups fed by various levels of silkworm pupae (T_1: with 100 % fish meal, T_2: 5 % silkworm pupae (SP) + 95 % fish meal, T_3: 10 % SP + 90 % fish meal, and T_4: 15 % SP + 85 % fish meal). *Bars* (mean ± SD) *with different letters* are significantly different ($p<0.05$)

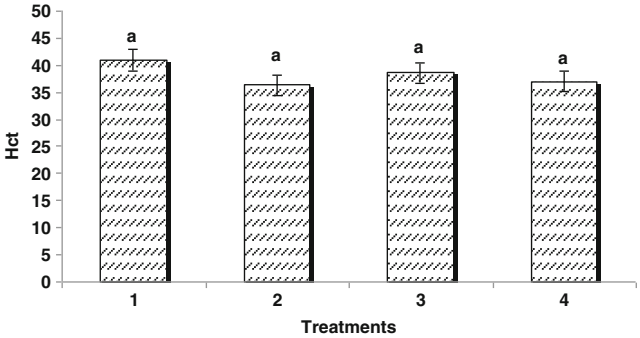

Fig. 3 Comparison of Hct percent between experimental fish groups fed by various levels of silkworm pupae (T_1: with 100 % fish meal, T_2: 5 % Silkworm pupae (SP) + 95 % fish meal, T_3: 10 % SP + 90 % fish meal, and T_4: 15 % SP + 85 % fish meal). *Bars* (mean ± SD) *with different letters* are significantly different ($p<0.05$)

Material and methods

The experiment was carried out for a period of 60 days at the Cold-Water Fish Research Center, Tonekabon, Iran. A total of 360 rainbow trout fingerlings (55±3.42 g) were randomly allotted to four treatment groups with three replicates of 30 fish per replicate including T_1: with 100 % fish meal, T_2: 5 % silkworm pupae (SP) + 95 % fish meal, T_3: 10 % SP + 95 % fish meal, and T_4: 15 % SP + 95 % fish meal. The fingerlings were distributed into 300 lit polyethylene tanks with stocking rate of 30 fish per tank. During the experiment, the water temperature was 16–18 °C, dissolved oxygen was 8–9 mg/L, and pH 7.3–7.5. The experimental diet was prepared according to the National Research Council recommendations for rainbow trout. To prepare the experimental diet, at first, all ingredients were pulverized and then mixed to homogenize. After that, the homogenized ingredients were mixed again with some 80 °C water for 30 min in order to shaping them. At the end, dry pellets with 3.5 mm in diameter were built by a pellet-making machine. A feeding size of 2.3 %/kg body weight was considered for daily feeding on basis of standard feeding schedule. Also, the daily feeding frequency during experiment period was calculated according to Takeuchi et al. (1978) as follow:

$$\text{Feeding frequency} = 40 \times \frac{\sqrt{W}}{T^{1/1}}$$

Where W refers to the total weight and T refer to water temperature (°C).

To determine the hematological parameters of serum, the blood samples were obtained from the caudal vein within 1 min using heparinized syringe at five times, i.e., 24, 48, 72, and 96 h after exposure to Butachlor. Before blood sampling, the fish were anesthetized using 100 ppm of MS222 (tricaine methane sulphonate), immediately after blood sampling, the blood samples were delivered to lab for red blood cell count (RBC), white blood cell (WBC), hematocrit (Hct), and hemoglobin (Hb) assays. The microhematocrit capillary tubes were used for measurement of hematocrit values

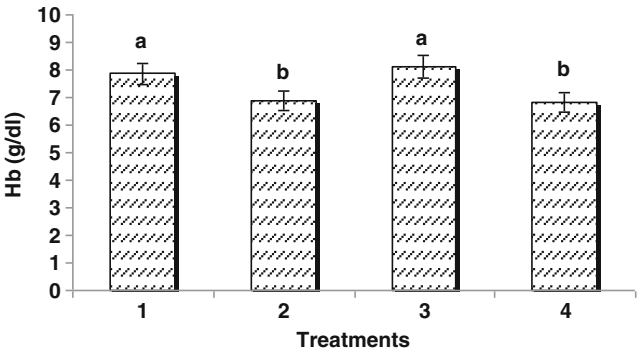

Fig. 2 Comparison of Hb concentration between experimental fish groups fed by various levels of silkworm pupae (T_1: with 100 % fish meal, T_2: 5 % Silkworm pupae (SP) + 95 % fish meal, T_3: 10 % SP + 90 % fish meal, and T_4: 15 % SP + 85 % fish meal). *Bars* (mean ± SD) *with different letters* are significantly different ($p<0.05$)

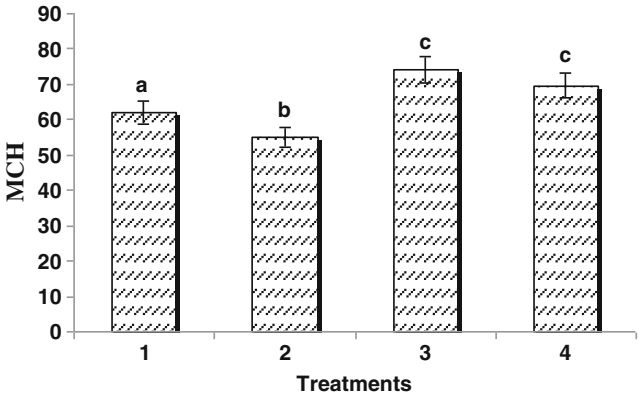

Fig. 4 Comparison of MCH (picograms) values between experimental fish groups fed by various levels of silkworm pupae (T_1: with 100 % fish meal, T_2: 5 % Silkworm pupae (SP) + 95 % fish meal, T_3: 10 % SP + 90 % fish meal, and T_4: 15 % SP + 85 % fish meal). *Bars* (mean ± SD) *with different letters* are significantly different ($p<0.05$)

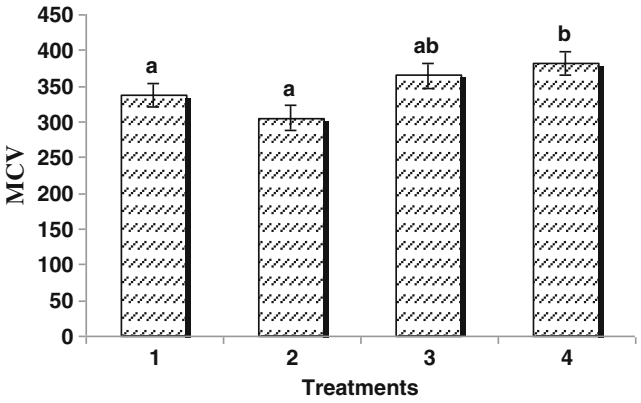

Fig. 5 Comparison of MCV (femtoliters) percent between experimental fish groups fed by various levels of silkworm pupae (T_1: with 100 % fish meal, T_2: 5 % Silkworm pupae (SP) + 95 % fish meal, T_3: 10 % SP + 90 % fish meal, and T_4: 15 % SP + 85 % fish meal). *Bars* (mean ± SD) *with different letters* are significantly different ($p<0.05$)

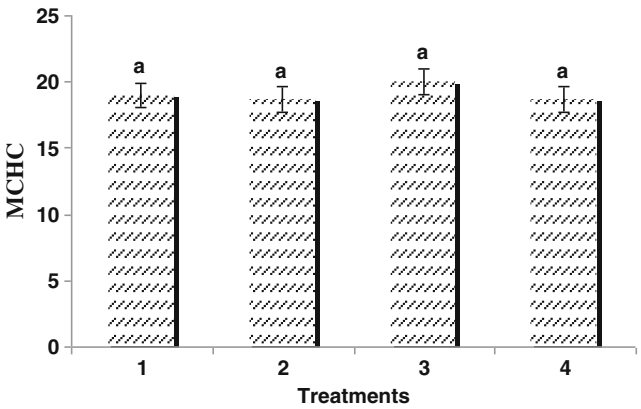

Fig. 7 Comparison of MCHC (grams per deciliter) values between experimental fish groups fed by various levels of silkworm pupae (T_1: with 100 % fish meal, T_2: 5 % Silkworm pupae (SP) + 95 % fish meal, T_3: 10 % SP + 90 % fish meal, and T_4: 15 % SP + 85 % fish meal). *Bars* (mean ± SD) with *different letters* are significantly different ($p<0.05$)

according to Rehulka (2003). The hemoglobin values were determined by cyanmethemoglobin according to Blaxhall and Daisley (1973). In this regard, the samples were centrifuged in order to avoid the variation in hemoglobin determination caused by the presence of nucleus from erythrocytes. An amount of 20 μl uncoagulated blood was mixed with 50 μl Drabkin's solution and then placed in dark environment for 5–10 min. Then, the hemoglobin concentration was measured by spectrophotometry in wavelength of 540 nm. RBC and white blood cells were determined with chamber method using Neubauers hemocytometer (Drabkin 1945).

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) values were calculated as follows, respectively:

$$\mathrm{MCV\ (fl)} = \mathrm{(hematocrit\ value)} \,/\, \mathrm{total\ number\ of\ RBCs\ (million\ mm^{-3})} \times 10$$
$$\mathrm{MCH\,(pg)} = \mathrm{(hemoglobin\ concentration)/total\ number\ of\ RBCs(million\ mm^{-3})} \times 10$$
$$\mathrm{MCHC\ (g/dl)} = \mathrm{(hemoglobin\ concentration)} \,/\, \mathrm{(hematocrit\ value)} \times 100$$

The SPSS software was used for data analysis. The percentage data were converted by angular transformation (arcsin √p) since these data did not have a normal distribution. One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which means were different.

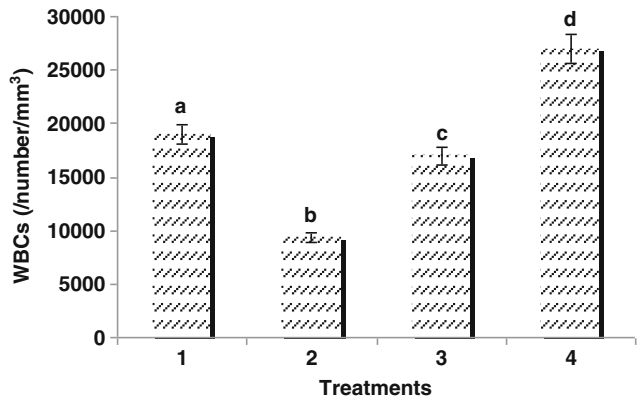

Fig. 6 Comparison of WBC count between experimental fish groups fed by various levels of silkworm pupae (T_1: with 100 % fish meal, T_2: 5 % Silkworm pupae (SP) + 95 % fish meal, T_3: 10 % SP + 90 % fish meal, and T_4: 15 % SP + 85 % fish meal). *Bars* (mean ± SD) *with different letters* are significantly different ($p<0.05$)

Results

After 60 days of experiment, the values of red RBCs (Fig. 1) and Hb (Fig. 2) were decreased significantly with increasing of silkworm pupae percent in diet ($P<0.05$). The lowest values of RBCs (990,000 RBC/mm^3) and Hb (6.83 g/dl) were observed in T_4 group. There were no significant differences between experimental treatments in terms of Hct values (Fig. 3, $P>0.05$). The MCH (Fig. 4), MCV (Fig. 5), and WBC (Fig. 6) values increased with increasing of silkworm pupae percent in diet ($P<0.05$). In this respect, the highest values of MCH (69.6 pg), MCV (382 fl), and WBCs (27,000 number/mm^3) were found in T_4 group. There were no significant differences between experimental treatments in terms of MCHC values (Fig. 7, $P>0.05$).

Discussion

Silkworm meal has been used in feeding monogastric and ruminant species for many years in Asian countries. In addition to this, the use of silkworm meal as a protein resource is expanding in aquaculture industry due to its cost-effectiveness. Silkworm pupae contribute to the good growth of the fish and increase the fish resistance to pathogens, as well as it is rich in calcium, vitamins, minerals, and other essential fatty acids. According to several studies, the hematological parameters of fish are affected by a range of factors, which include species, size, age, physiological status, environmental conditions, and dietary regime, e.g., quality and quantity of food, dietary ingredients, protein sources, and vitamins (Houston 1997; Osuigwe et al. 2005).

In the present study, we investigated the changes of hematological parameters of rainbow trout in response to dietary levels of silkworm pupae. According to results, the values of WBCs elevated significantly in response to increasing of silkworm pupae levels in diet. Generally, increases in WBCs are usually an immunity reaction to pathogens. Elevated levels of WBCs in response to silkworm pupae incorporation may be due to some components in silkworm pupae that stimulate fish immune system (Ijayva and Eko 2009). In our study, the RBC and Hb concentrations reduced with increasing silkworm pupae percent in diet. It is obvious that with the decreasing RBCs, the values of MCV (hemoglobin concentration/RBCs) decreases as MCV values decreased with reduction of RBCs in the present study. In the present study, the significant decrease in RBCs could be in relation to inhibitory action of some components of silkworm pupae on hematopoietic organs (Ijayva and Eko 2009), as well as it is likely that this components decrease RBCs by lysis of them in blood (Ijayva and Eko 2009). In conclusion, our study showed that silkworm pupae stimulate the rainbow trout immune system as WBCs increased in response to dietary silkworm pupae. On the other hand, dietary silkworm pupae can cause some signs of anemia as the values of RBCs and Hb decreased after use of this protein resource.

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