

Effects of *Aloe vera* extract on growth and some hematological parameters of shirbot, *Tor grypus* (Heckel, 1843)

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Abstract

A feeding trial was conducted for sixty days to determine the effect of dietary *Aloe vera* on parameters related to growth rate, health status and hematological parameters of shirbot (*Tor grypus*). Two hundred and forty fish weighing 50-60 g were randomly subjected to four different treatments, including a control, T1 (0.1% of *A. vera*), T2 (0.2%) and T3 (0.5%) in triplicate. Hematological parameters and some growth parameters, including the body weight, total length, condition factor, feed conversion ratio, feed efficiency, specific growth rate and protein efficiency ratio were measured. Administration of fish to different concentrations of *A. vera* extract led to significant ($p < 0.05$) increase in total erythrocyte count, packed cell volume, and hemoglobin when compared with the control group. Mean corpuscular hemoglobin concentration were increased only in T2 and T3 in comparison with the control. When animals received 5% of *A. vera* daily, they showed a significant increase (29%) in white blood cells ($p < 0.05$). Growth parameters were improved with the addition of different concentrations of *A. vera* to fish food. According to the results obtained, it might be concluded that feeding this species with *A. vera* extract could likely enhance growth rate and also hematological parameters.

Keywords: *Aloe vera*, Growth parameters, Hematological indices, *Tor grypus*

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Introduction

Fish pathogenic organisms are serious threats to economic viability of any aquaculture practice. Currently, the use of antibiotics for prophylaxis and treatment of diseases leads to the development of antibiotic resistant bacterial strains, accumulation of residue in cultured fish and environmental problems. Therefore, a new approach to immunotherapy is actively used to prevent or cure fish diseases. In this regard, extensive research has been carried out to test various immunostimulants including medicinal plants which have been found to be effective in fish. It has been found that to use medicinal herbs in fish diets enhances the immune system against infections with various bacteria (Castro *et al.*, 2008; Ahmad *et al.*, 2011; Maqsood *et al.*, 2011; Begum and Navaraj, 2012)

The diversity of plant species in Iran can be observed due to the variety of weather conditions that might provide the availability of more natural pharmaceuticals and poison plants to people throughout the country. Application of plants is a very ancient medicinal treatment. Thousands of plant species grow in Iran with different kinds of pharmaceutical properties.

The *Tor gryp* is one of the most important fish species in southwest Asia (Iran, Iraq, Turkey and Syria) due to its excellent biological characteristics such as fast growth and high resistance against natural stressors, marketable taste, and high economic value.

Aloe barbadensis Miller (*Aloe vera*), is a perennial plant of the lily (Liliaceae) or Aloeaceae family, which is a tropical or subtropical plant characterized by lance-shaped leaves with jagged edges and sharp points (Alishahi *et al.*, 2010). More than

500 species of *A. vera* are known. This plant is native to tropical areas, including the north of Africa, Europe and the southern part of the Mediterranean region (Grindlay and Reynolds, 1986). *A. vera* grows widely in the south of Iran and is cultured for the pharmaceutical uses. The only species of *A. vera* that can grow in Iran is *A. littoralis baker* that is seen in the southern area and islands of the Persian Gulf (Mazaffarian, 1996; Soltanipoor, 2006). Aloe inner gel is the colorless gel consisting primarily of water and polysaccharides, including pectin, cellulose, hemi cellulose, glucomannan, acemannan and mannose derivatives (Lee *et al.*, 2001). Acemannan is considered to be the main functional component of *Aloe vera* and is composed of a long chain of acetylated mannose (Lee *et al.*, 2001). Among the health benefits of *Aloe vera* leaves the acceleration of ulcer healing, immune stimulating, antiviral and anticancer effects can be mentioned (Waihenya *et al.*, 2002). Besides the problem of high costs of fish feeds and quality seed, disease outbreak is a major challenge in fish farming (Ayoola *et al.*, 2013), and herbal care was improved to restrain the most injurious parasitic illnesses infecting humans, animals and fishes. In this study, the effects of dietary *A. vera* were investigated in shirbot (*T. grypus*) in order to discover its effects on growth parameters and hematological indices.

Materials and methods

Fish and water sources

A total number of 240 pond reared shirbot, *T. grypus*, with an average body weight of 50-60 g, were obtained from a fish farm in

Dezful, Khuzestan Province, Iran. Fish were transferred to our laboratory and were kept in plastic tanks. All fish were then adapted to laboratory conditions within one week prior to the experiment. Water quality parameters, including temperature (25 °C), dissolved oxygen (8-10 ppm), pH (7.9), NO₂ (<0.01 ppm) and NH₃ (<0.1 ppm) were recorded daily during the experiment. Only 10% of total water was exchanged daily to reduce the risk of metabolic toxicosis.

Diet preparation

The experimental diets were prepared by mixing of normal shirbot food with crude extract of *A. vera* (Baridj essence product, Kashan, Iran). For better homogenization, one volume of the crude extract of *A. vera* was dissolved in 5 volumes of water and the homogenized solution was then sprayed at the rate of 0.1%, 0.2% and 0.5% onto a thin layer of food. The *A. vera*-free diet was sprayed by the same method with only water.

Fish groups and treatments

As mentioned earlier, following the acclimation period, 240 fish were selected and were randomly distributed into twelve tanks, three replicates for each treatment (i.e. 20 fish were maintained in each 100 L tank), which were equipped with a thermostatic heater, aeration and external biofilters. All fish were fed with *Aloe vera*-treated diets and another group was fed with an *A. vera* free diet (Control). The 4 groups were named as follows:

Group 1: Control

Group 2(T1): 0.1% *A. vera*

Group 3(T2): 0.2% *A. vera*

Group 4(T3): 0.5% *A. vera*

All treatments were fed twice daily at 5% body weight during the experimental period (2 months).

Growth parameters measurements

The recorded data for weight and length were used for calculation of weight gain (%), protein efficiency ratio (PER), feed conversion ratio (FCR), specific growth rate (SGR), total length (TL) and condition factor (CF) for each group using the following equation (Sales-Leiton *et al.*, 2010):

WG (%) = [(final weight - initial weight) / initial weight] × 100

SGR (%) = [ln final weight - ln Initial weight / time interval in days] × 100

FCR = [feed given (dry weight) / weight Gain (wet weight)]

TL = (Final length) - (Initial length)

CF = [weight gain / total length³] × 100

PER = net weight gain (weight gain) / protein fed

Blood collection

At the end of the experiment, the fish were anaesthetized using MS222, then weighted and measured. Blood samples of 5 fish in each treatment were collected from the caudal vein.

Haematology

The blood samples were transferred to glass tubes and hematological parameters were immediately determined. Total leucocytes count and total erythrocyte count were determined by using the Neubauer counting chamber as described by Schaperclaus *et al.* (1991) and packed cell volume (PCV) was determined by centrifugation at 2000 rpm for 20 min. Haemoglobin (Hb) concentration was

determined according to the cyanomethaemoglobin procedure (Goldenfarb *et al.*, 1971). Nonclotted blood (0.02 cc) was diluted with 5 cc of Drabkin solution and left to stand for 10 min at room temperature. The absorbance was read at 540 nm and the amount of hemoglobin was calculated against a hemoglobin standard. Mean corpuscular volume (MCV), mean corpuscular haemoglobin content (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated well (Hu *et al.*, 2005).

$MCV (\mu m^3 \text{ cell}^{-1}) = (PCV \text{ as percentage/RBC in millions cell } mm^3) \times 10$
 $MCH (pg \text{ cell}^{-1}) = (Hb \text{ in g } 100 \text{ ml}^{-1}/RBC \text{ in millions cell } mm^3) \times 10$

$MCHC (g \text{ } 100 \text{ ml}^{-1} \text{ PCV}) = (Hb \text{ in g } 100 \text{ ml}^{-1}) \times 100$

Statistical analysis

The statistical difference between each treatment and their replicates were assessed using one-way analysis of variance (ANOVA) techniques followed by Duncan's multiple range test using

statistical package (SPSS 18.0) to find out the significant difference at 5% level ($p < 0.05$) of significance.

Results

Growth

The growth performance of shirbot, *T. grypus*, in terms of percentage weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF), protein efficiency ratio (PER) and feed efficiency (FE) are presented in Table 1.

The obtained results showed that PER and FE in different treatments were significantly ($p < 0.05$) increased when compared with the control. The FCR and CF in different treatments were reduced in comparison with the control group. The WG and SGR in T2 were significantly ($p < 0.05$) higher than both control and other *A. vera* treatments following 60 days. Other treatments showed either significant and/or insignificant increase in WG and SGR compared with the control.

Table 1: Growth parameters of *Tor grypus* fed with different *Aloe vera* diets

Parameters	Treatments			
	Control (Treatment 1)	0.1% <i>Aloe vera</i> (Treatment 2)	0.2% <i>Aloe vera</i> (Treatment 3)	0.5% <i>Aloe vera</i> (Treatment 4)
CF	1.02±0.03 ^a	0.84±.17 ^b	0.81±0.24 ^b	0.72±0.06 ^b
WG	17±3.00 ^c	29±6.00 ^b	50±7.00 ^a	20±1.00 ^{bc}
SGR	0.26±0.05 ^c	0.42±0.09 ^b	0.68±0.08 ^a	0.31±0.01 ^{bc}
PER	0.52±0.10 ^b	1.69±0.30 ^a	1.84±.30 ^a	1.53±.10 ^a
FCR	4.89±0.65 ^a	1.51±0.27 ^b	1.38±0.25 ^b	1.64±0.08 ^b
FE	0.21±0.02 ^b	0.68±0.12 ^a	0.74±0.12 ^a	0.61±0.03 ^a

Values (mean±SD) with different letters in the same line indicate significant differences ($p < 0.05$)

Hematological parameters

Among the hematological parameters, total erythrocyte count, total leukocyte count,

haemoglobin, PCV have been significantly ($p < 0.05$) increased following different *A. vera* treatments compared with the control

that are shown in Figs. 1, 2, 3 and 4. MCHC in T2 and T3 were only different from the control group ($p < 0.05$) (Fig. 7). No significant ($p > 0.05$) differences in MCV, MCH between all treatments, including different *A. vera* extract and control group were found (Figs. 5, 6). The effect of *A. vera* on different white blood

cells, including eosinophil, basophil, heterophil and lymphocyte was also measured. The most leukocyte, observed in different treatments, was related to lymphocyte. Heterophil and eosinophil values were not affected by dietary *A. vera* after 60 days (Fig. 8).

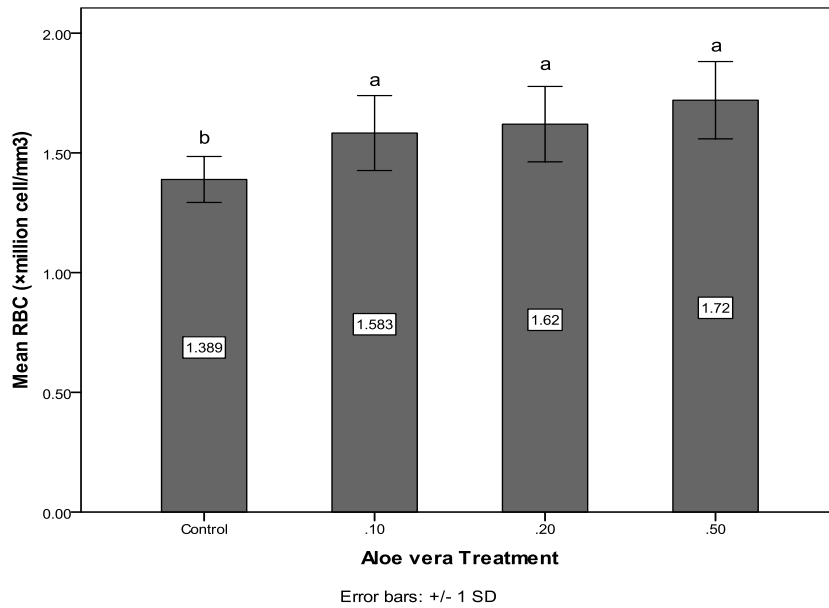


Figure 1: The effect of *Aloe vera* crude extract on total RBC count. Parameters with significant differences ($p < 0.05$) are marked by different alphabetic letters.

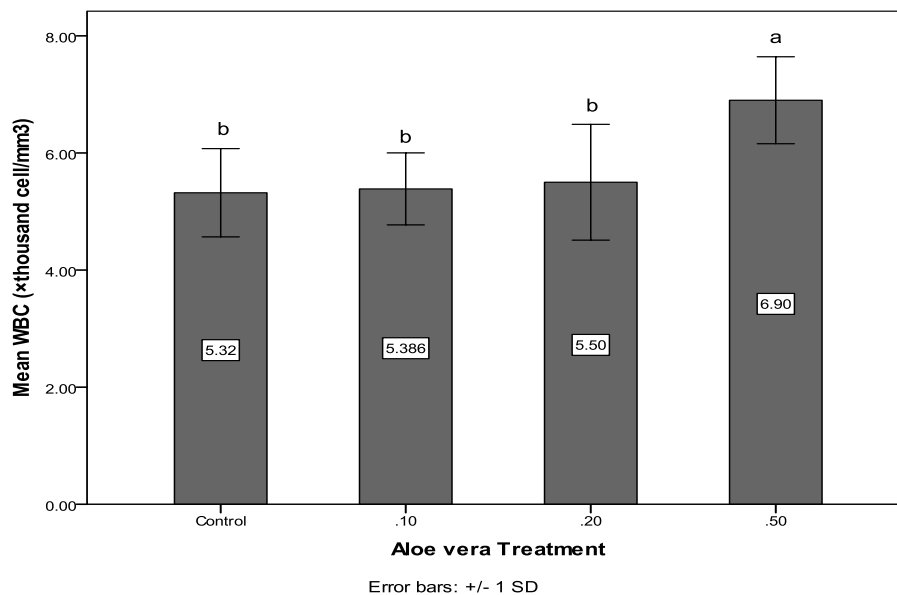


Figure 2: The effect of *Aloe vera* crude extract on total WBC count. Parameters with significant differences ($P < 0.05$) are marked by different alphabetic letters.

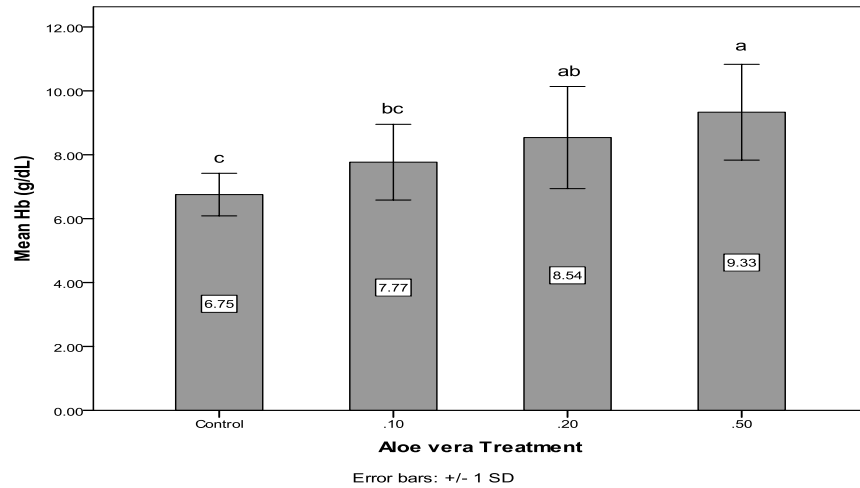


Figure 3: The effect of *Aloe vera* crude extract on Hb. Parameters with significant differences ($p < 0.05$) are marked by different alphabetic letters.

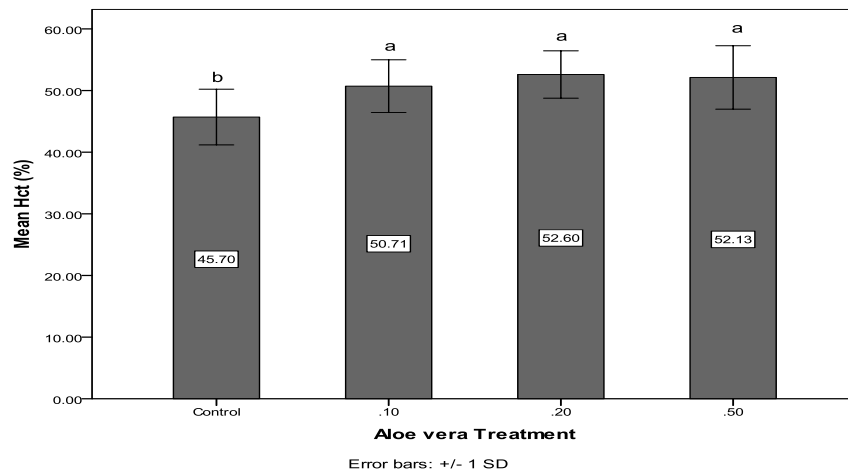


Figure 4: The effect of *Aloe vera* crude extract on PCV. Parameters with significant differences ($p < 0.05$) are marked by different alphabetic letters.

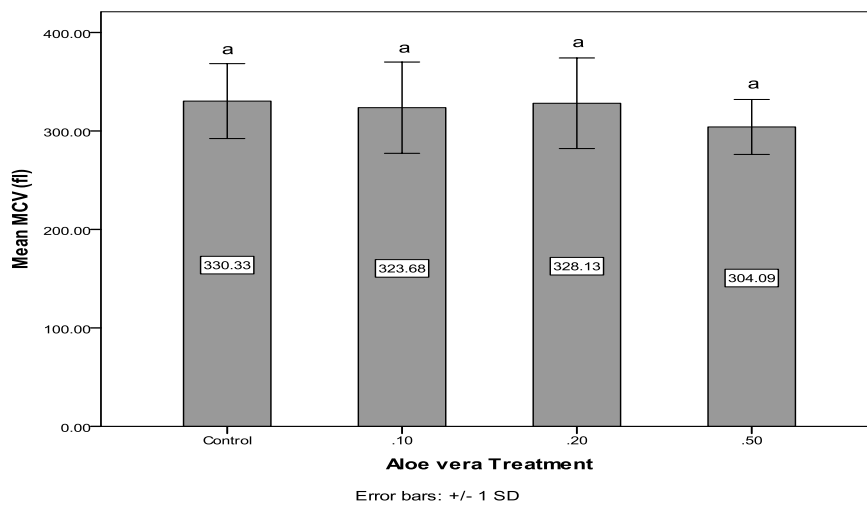


Figure 5: The effect of *Aloe vera* crude extract on MCV. Parameters with significant differences ($p < 0.05$) are marked by different alphabetic letters.

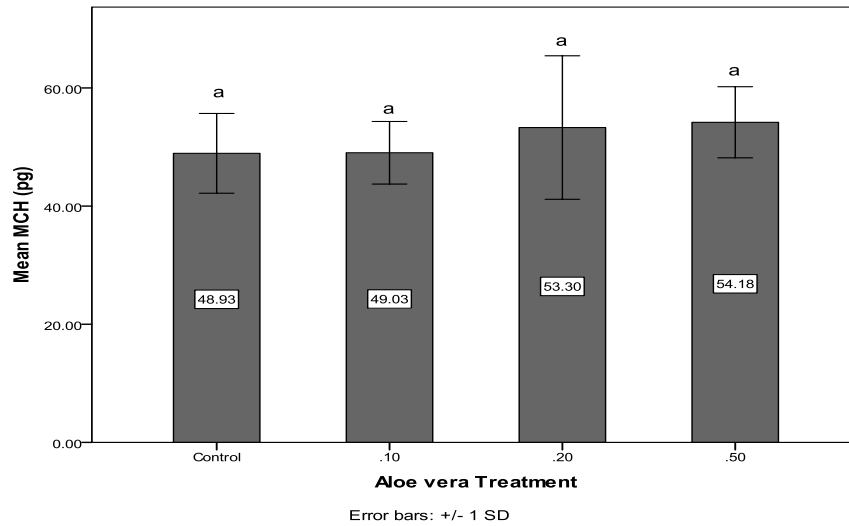


Figure 6: The effect of *Aloe vera* crude extract on MCH. Parameters with significant differences ($p < 0.05$) are marked by different alphabetic letters.

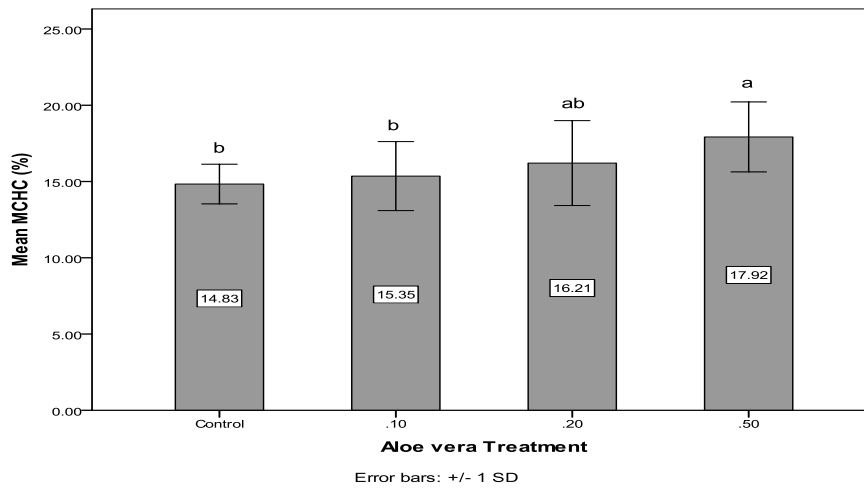


Figure 7: The effect of *Aloe vera* crude extract on MCHC. Parameters with significant differences ($p < 0.05$) are marked by different alphabetic letters.

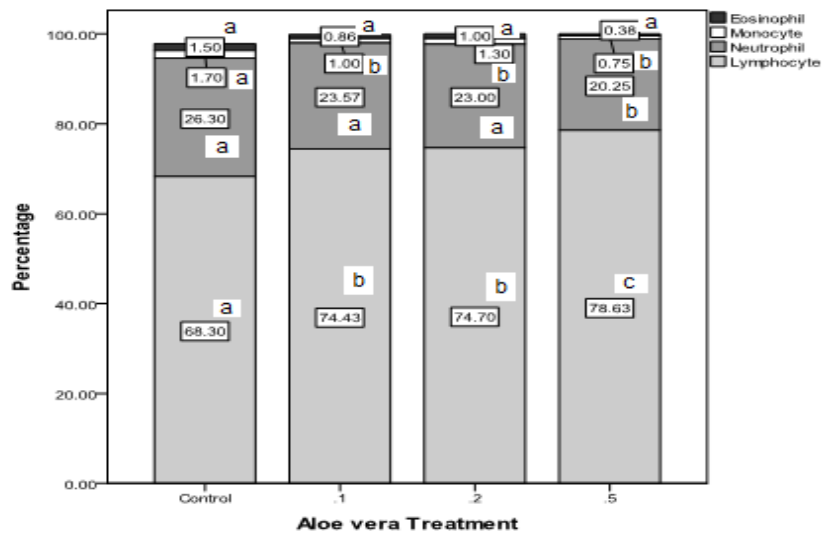


Figure 8: The effect of *Aloe vera* crude extract on different white blood cells. Parameters with significant differences ($p < 0.05$) are marked by different alphabetic letters.

Discussion

The aim of this study was, investigating effects of *A. vera* extract on growth and some hematological parameters of *T. grypus*. Fish exist in lower levels of evolution; therefore, their immune system is simpler and more elementary than warm-blooded animals. Nowadays, a trend to the use of immune stimulants has been developed. For various reasons, including health, environmental and economic defects of antibiotics, vaccine inefficiency in aquatic animals and efficiency of innate immunity in fish, the application of herbal stimulants in aquatic animals is preferred (Alishahi, 2004). In recent years the immune stimulants in aquatic animals have been considered (Secombes and Yano, 1996). Among the immune stimulants, herbal stimulants have notable advantages and attitude to use them have been developed (Jain and Wu, 2003; Dügenci *et al.*, 2003). The effects of the immune stimulants on growth parameters in fish are reported frequently (Raa, 1996), for example β -glucan in combination with lipopolysaccharide (Selvaraj *et al.*, 2006), chitosan (Gopalakannanand Arul, 2006), levamisole (Alvarez *et al.*, 2006) and ergosan (Gioacchini and Arul 2008). Results of the present study showed that oral administration of *Aloe vera* had significant ($p < 0.05$) effects on hematological parameters of shirbot, *Tor grypus*. Total erythrocyte count, PCV, Hb in different groups and MCHC in T2 and T3 were significantly different ($p < 0.05$) compared with the control group. MCV and MCH were not significantly different even after 60 days feeding with *Aloe vera* ($p > 0.05$). This attribute observed in *Aloe vera* gel may be due to the presence of

thiamine, riboflavin, folic acid and other essential and non-essential amino acids in *Aloe vera* (Hamman, 2008). The polysaccharides, which are the major component of *Aloe vera*, have also been reported to stimulate erythropoiesis (Choi and Chun, 2003; Ni *et al.*, 2004). Various studies have been conducted on the effects of the immunostimulants on hematological parameters, and different results have been reported. Some of the scientists reported that the immune stimulants are effective (Kajita *et al.*, 1990; Marian, 2004), while some others reported that the immunostimulants are not effective on hematological parameters (Sakai, 1999; Alishahi *et al.*, 2010; Farahi *et al.*, 2012). Results in the present study showed that *A. vera* extract with unknown mechanisms can stimulate hematopoiesis. Mesbah *et al.* (2008) investigated the effects of the *A. vera* extract in carp and reported that *A. vera* had no adverse effects on total erythrocyte count, PCV and complement activity. However, results showed that surviving rate in treatments, total leukocyte count, antibody titer against *Aeromonas*, lysozyme activity and bactericidal activity had increased compared with the control group ($p < 0.05$). Usually, total leukocyte count increases after environmental stresses and attack of pathogens. Sometimes an increase in leukocytes indicates that immunity has increased.

Improvement of growth parameters following *A. vera* administration in common carp have been reported previously (Mesbah *et al.*, 2008). In another experiment, done by Alishahi (2010) the effect of *A. vera* in *Amphilophus labiatus* was investigated. The

author reported that the concentrations 0.5 and 1% of *A. vera* led to significant improvements in weight gain (WG), food conversion ratio (FCR) and specific growth rate (SGR) ($p < 0.05$). Results of the present study showed that oral administration of *A. vera* extract had significant ($p < 0.05$) effects on growth parameters of shirbot, *T. grypus*, every two weeks after feeding as compared to the placebo group. These results are consistent with the results obtained by Heidarieh *et al.* (2013) who reported enhanced growth performance, gastrointestinal and skin morphology in rainbow trout treated with dietary *A. vera*. This data also supports the study of Mahdavi *et al.* (2013) who reported better final length and weight and growth indicators in treatments fed with *Aloe vera* (Heidarieh *et al.*, 2013; Mahdavi *et al.*, 2013). Conversely, *A. vera* at different inclusion rates had no effect on *Acipenser baerii* (Wang *et al.*, 2011).

In conclusion, the results obtained showed that the concentrations 0.2 and 0.5% had better effects on growth and hematological parameters of shirbot, *T. grypus*. Therefore, we suggest that the addition of 0.2% extract is economically better than other concentrations. Although there were no significant differences between 0.2 and 0.5% *A. vera* extract, a lower concentration is better from an economical viewpoint and biosecurity.

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