

Study on nanoparticles of *Aloe vera* extract on growth performance, survival rate and body composition in Siberian sturgeon (*Acipenser baerii*)

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Abstract

The application of nanotechnology in drug delivery and a variety of supplements is very impressive. In this regard, the use of this knowledge can be effective in the performance of medicinal plants in aquaculture. In the present study *Aloe vera* was extracted and its nanoparticles were produced. Three levels of *Aloe vera* nanoparticles in Siberian sturgeon (*Acipenser baerii*) were evaluated for the effects on growth performance, survival rate and body composition. A total of 360 Siberian sturgeon (*A. baerii*) with an average of 10.95 ± 0.04 (g) randomly divided into four treatment groups with three replicates each. The treatment groups were fed with diets that included 0% (control), 0.5, 1 and 1.5% of *Aloe vera* nanoparticles for 60 days. Weight gain, initial body weight, condition factor, feed conversion ratio, specific growth rate, protein efficiency ratio, survival rate, and body composition (protein, lipid, carbohydrate, ash and moisture) were measured and compared among the fish in the different groups. The results showed that growth indices of fish fed the *Aloe vera* nanoparticles were higher than the control diet, so that the difference between the control group and 1% *Aloe vera* nanoparticles was more than that in other treatments. In each of the body composition parameters, no significant differences were observed among the treatments and control groups ($p > 0.05$). Considering that there are no significant differences in results between 1% and 1.5% *Aloe vera* nanoparticles, the results showed that adding 1% *Aloe vera* nanoparticles to the fish diet improves the growth factors of Siberian sturgeon.

Keywords: *Aloe vera* extract, Nanoparticles, Growth, Body composition, *Acipenser baerii*

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Introduction

In recent years, preparing nanoparticles for drug delivery is regarded as one of the research areas in new methods of drug delivery. One of the favorite characteristics of drug nanoparticles is their biocompatibility so that they can adapt to the body of the organism without causing harm. Today it is proved that the drug delivery systems based on nanoparticles are more effective and have less side effects, more acceptability and more accumulation in the considered site (Douglas *et al.*, 1987). Therefore, in view of this science it is very important to make use of the advantages of this science to improve productivity in aquaculture.

During the past two decades, much success in the use of medicinal plants has been achieved in the aquaculture industry (Ham *et al.*, 2001). Siberian sturgeon (*Acipenser baerii*) is an anadromous fish and is imported into Iran. It is easily adapted to the conditions of breeding and resistant to changes in environmental conditions (Pyka and Kolman, 2003). Fast growth, short sexual maturity and caviar production in a short time, the wide range and variety in the diets are the various reasons that this species has been introduced for farming, in freshwater to produce sturgeon meat (Adamek *et al.*, 2007). Considering that aquaculture development is influenced by various environmental factors and nutrition are placed, so herbal additives can affect indicators such as

digestibility, nutrition effectiveness and food taste, to affect the growth in aquaculture.

There has been published many studies which confirmed that the addition of plants and their extracts in the diets has a beneficial effect to improve growth parameters and protect from diseases in aquaculture (Shalaby, 2004; Sasmal *et al.*, 2005; Johnson and Banerji, 2007; Hajibeglou and Sudagar, 2010; Farahi *et al.*, 2012).

Aloe vera (synonym: *Aloe barbadensis* Miller) belonging to the family liliaceae is widely distributed in the tropical and subtropical regions of the world. Most of *Aloe* species are indigenous to Africa, but now have wide distribution in the tropical and subtropical regions of the world (Mahdavi *et al.*, 2013).

Acemannan and the other constituents of *A. vera* have been found to improve macrophage activity as much as tenfold, enhance macrophage effectiveness in modulating the entire immune system, and to stimulate, produce, and release antibodies (Haghighi *et al.*, 2014). Despite numerous studies on the therapeutic effects of this plant in warm-blooded animals, few studies concerning the effects of immune stimulation and growth in fish have been executed (Alishahi *et al.*, 2010).

Because no study has been conducted on nonmetal nanoparticles in transferring natural material such as herbal compounds to fish diets up to now, the present study evaluates the

effects of three doses of *A. vera* nanoparticles on growth parameters, survival rate and body composition of this valuable species (Siberian sturgeon).

Materials and methods

Preparation of Aloe vera extract

The plant of *A. vera* was procured from Medicinal Plants Production Cooperation of Havin (Havin Co.). The leaves were collected and washed in sterile distilled water and evacuated from gel. The leaves were separately shade-dried. The extract was prepared with the standard method of percolation. This crude extract was stored at -18°C until use (Ozakan *et al.*, 2007; Haghighi *et al.*, 2014).

Preparation of Aloe vera nanoparticles

In order to create *A. vera* nanoparticles, the produced extract was transferred to the Pharmaceutics Lab of School of Pharmacy in Zanzan University of Medical Sciences. Nanoparticles were produced according to patent no.73360 (Hamidi *et al.*, 2011). In order to measure size of particles and confirm them to be nanoparticles, and also to calculate electric charge in them, Zetasizer Set was used (Fig. 1). The diameter of *A. vera* nanoparticles is 152nm and PDI=0.25 (Poly dispersity Index). After confirming the size of nanoparticles, cryoprotector was added to nano extract suspension at 40° C to increase preservation time and change it to a powdery extract.

At the end of the process of producing *A. vera* nanoparticles, gas chromatography (GC-MS) and liquid chromatography (HPLC) were used to analyze compounds in *Aloe vera* extract (Table 1) (Rouessac and Rouessac, 2007; Lakshmi and Rajalakshmi, 2011).

Experimental food preparation

In order to produce food containing different percentages of *A. vera* nanoparticles (0.5%, 1% and 1.5%), Biomar commercial plate containing 49% Crude protein, 15% Crude lipid, 2.5% cellulose, 1.24% phosphorus, 8.7% ash, 1.85% calcium and 0.55% sodium was applied. To add extract powder and prepare food diets containing different amounts of *A. vera* nanoparticles, the method of dissolving the powder in water and spraying the extract on the food was used and then the levels of these compounds were stirred in.

The prepared foods were dried on separated trays at room temperature for 24hr. Then, oil was sprayed on the dried food according to a standard method (Noga, 2000). The prepared food was placed in special closed dishes and preserved in 4-6°C for distribution in different feeding times.

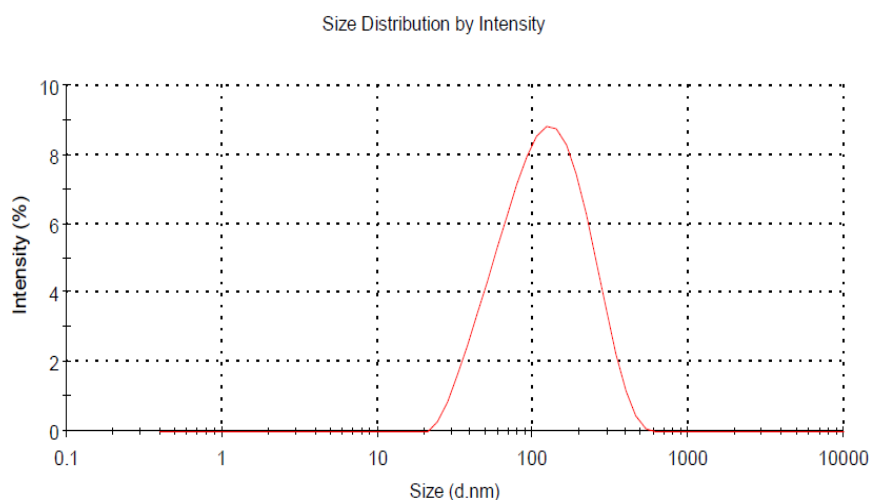


Figure 1: Size distribution by intensity.

Table 1: Types and amounts of compounds in *Aloe vera* nanoparticles.

Compounds	Amount (%)	Compounds	Amount (%)
Alolin	28.7	Comaric acid	7.59
Oleic acid	5.98	Squalene	13.7
B-Sitostrol	1.4	Limoene	9.12
Lupeol	4.7	n- Hexadecanoic acid	10.22
Campestrol	2.06	other components	8.14
Carvone	8.39		

Experimental design

The study was carried out at International Sturgeon Research Institute, Rasht, Iran. A total of 360 Siberian sturgeons (average weight of 10.95 ± 0.04 g) were randomly divided into 12 tanks of dewatering volume 350 liters. Fishes were randomly divided into 4 groups (3 treatments and one control group) in 3 replications, each containing 30 fish. After the adaptation period, the fish were kept in a controlled condition (Temperature = $22.8 \pm 0.88^\circ\text{C}$, pH = 6.8 ± 0.19 and dissolved oxygen = 6.74 ± 0.42 mg/L). Environmental parameters and survival were controlled daily. During the two-

month breeding, prepared foods containing nanoparticles of *A. vera* extract (0.5%, 1% and 1.5%) and Biomar normal diet (for control group) at a rate of 3% of body weight were fed at 8:00, 15:00 and 22:00 hours.

Growth performance

In order to measure the growth indices of the Siberian sturgeon, biometry was done in all groups once every two weeks during the experiment. After two months, all of the fish in experimental groups were weighed using a digital scale (0.01 g). Weight Gain (WG), Initial body weight (IBW), Specific growth rate (SGR), feed conversion

ratio (FCR), condition factor (CF), Protein efficiency ratio (PER) and Survival rate (SR) were calculated using standard formula (Lee and Kim, 2001; Tachjian *et al.*, 2006; Mohseni *et al.*, 2014).

Weight Gain (WG) = $W_f - W_i$

Initial body weight (IBW) = $(W_f - W_i) \times 100 / W_i$

Specific growth rate (SGR) = $100 \times (\ln W_f - \ln W_i) / \text{Days}$

Feed conversion ratio (FCR) = Food intake/Weight gain

Condition factor (CF) = $(W/L^3) \times 100$

Protein efficiency ratio (PER) = $(W_f - W_i) / \text{protein intake}$

Survival rate (SR) = $[(N_f - N_i) / N_i] \times 100$

Where, \ln is natural logarithm, W_f is mean final weight (g) and W_i is mean initial weight (g)

Body composition

At the end of the two month feeding period, to assess changes in body composition, levels of protein, lipid, carbohydrate and ash were measured in different treatments and the control group. Three fish from each replicate (total 36) were randomly selected and then testing carcass analysis was performed according to the AOAC (2000) method. Protein content was measured using the Kjeldahl method. Laboratory methods were used to measure lipid content using ether solvents. Moisture was determined by putting the sample in an oven at 105°C and weighing it after drying in a desiccator. The ash samples were burned in a furnace at a temperature of 550°C for 5 hours and were weighted. The carbohydrate rate was obtained by subtracting the sum numbers of protein, lipid, moisture and ash from 100.

Statistical analysis

Statistical analyses were performed using SPSS (version 20) software. Data are presented as mean±SE. All the data were tested for normality (Kolmogorov-Smirnov test). Data were analyzed by one-way variance analysis (ANOVA). The significant means were compared by Duncan's test and a $p < 0.05$ was considered statistically significant.

Results

The results of growth parameters in fish fed with *A. vera* compared with the control group are presented in Table 2.

Based on the results the highest and the lowest total weights in Siberian sturgeon were observed in the group fed 1.5% *A. vera* nanoparticles and the control group, respectively. Weight gain in the 1.5% and 1% *A. vera* nanoparticles were statistically significant compared to that in the control group ($p < 0.05$). Also, the highest and lowest FCR was found in the control group and 1.5% *A. vera* nanoparticles, respectively. In addition, there were statistically significant differences between treatments and the control group ($p < 0.05$). The highest amounts of WG, SGR, IBW and PER were observed in the 1.5% *A. vera* group and lowest amounts were found in the control group.

Table 2: Growth performance in Siberian sturgeon fed with different levels of *Aloe vera* nanoparticles (mean \pm SE)

Parameters/treatments	Control	0.5%	1%	1.5%
Initial weight (g)	10.98 \pm 0.02 ^a	10.85 \pm 0.04 ^a	10.97 \pm 0.06 ^a	11.05 \pm 0.05 ^a
Final weight (g)	62.4 \pm 0.28 ^a	83.79 \pm 0.59 ^b	98.02 \pm 0.24 ^c	98.82 \pm 0.21 ^c
Initial length (cm)	15.61 \pm 0.02 ^a	15.51 \pm 0.06 ^a	15.52 \pm 0.09 ^a	15.6 \pm 0.06 ^a
Final length (g)	28.04 \pm 0.05 ^a	30.51 \pm 0.19 ^b	31.52 \pm 0.11 ^c	31.7 \pm 0.07 ^c
weight gain (g)	51.42 \pm 0.19 ^a	72.94 \pm 0.46 ^b	87.05 \pm 0.39 ^c	87.77 \pm 0.41 ^c
SGR (%)	2.89 \pm 0.003 ^a	3.4 \pm 0.016 ^b	3.64 \pm 0.012 ^c	3.65 \pm 0.09 ^c
FCR (%)	1.28 \pm 0.007 ^c	0.94 \pm 0.009 ^b	0.88 \pm 0.003 ^a	0.87 \pm 0.003 ^a
Initial body weight (%)	468.13 \pm 2.52 ^a	672.33 \pm 7.54 ^b	793.19 \pm 6.92 ^c	794.16 \pm 5.48 ^c
condition factor	0.28 \pm 0.003 ^a	0.29 \pm 0.004 ^b	0.31 \pm 0.004 ^c	0.31 \pm 0.002 ^c
Protein efficiency ratio	1.27 \pm 0.14 ^a	1.71 \pm 0.2 ^b	2 \pm 0.25 ^c	2.01 \pm 0.11 ^c
Survival rate (%)	100	100	100	100

Differences in Latin letters in each row shows a significant difference ($p < 0.05$).

CF amounts in all treatments containing *A. vera* nanoparticles were at one level and the lowest rate was observed in the control group. In addition, a significant differences were seen between treatments and control group ($p < 0.05$). According to Table 2, the maximum and minimum amount of PER were observed in the 1.5% *A. vera* nanoparticles group and the control group, respectively. Significant differences were shown between treatments ($p < 0.05$). During the period of two months, mortality was not observed and survival in the treatments and control group was 100%.

In each of the body composition parameters, no significant difference was observed between the treatments and control groups ($p > 0.05$). However, the amounts of carbohydrate, ash and

moisture in treatments were lower than that in the control group (Table 3).

The highest and lowest rate of body protein was observed in the 1.5% *A. vera* treatment and control group, respectively. Also, the highest and lowest rate of lipid was seen in the 0.5% treatment and control group, respectively, whereas, the highest rates of carbohydrate, moisture and ash were measured in the 1%, 0.5% and 1.5% treatments, respectively.

Table 3: Body composition amounts in Siberian sturgeon fed with different levels of *Aloe vera* nanoparticles (mean \pm SD).

Parameters /treatments	Control	0.5%	1%	1.5%
Protein %	18.34 \pm 0.14 ^a	18.86 \pm 0.21 ^a	19.55 \pm 0.12 ^a	19.67 \pm 0.03 ^a
Lipid %	3.56 \pm 0.08 ^a	4.8 \pm 0.13 ^a	4.39 \pm 0.11 ^a	4.31 \pm 0.09 ^a
Carbohydrate %	0.81 \pm 0.02 ^a	0.44 \pm 0.02 ^a	0.34 \pm 0.01 ^a	0.39 \pm 0.03 ^a
Ash %	2.15 \pm 0.06 ^a	1.55 \pm 0.06 ^a	1.45 \pm 0.07 ^a	1.2 \pm 0.04 ^a
Moisture %	75.14 \pm 0.04 ^a	74.15 \pm 0.06 ^a	74.27 \pm 0.11 ^a	74.43 \pm 0.19 ^a

Means with the same superscript letter at the same row are not significantly different ($p>0.05$).

Discussion

Regarding the advantages of applying medicinal plants in different areas, particularly for the prevention and cure of fish diseases, it seems that using new sciences and technologies can be efficient for better use of herbal compounds. So, considering the potential of nanoparticles to transfer nutrition compounds, it can be efficient to use this technology to convey herbal compounds to the fish body.

Recently, due to the effective compounds found in herbs, they have received increased attention in aquaculture attention to improve growth and increase the survival rate (Abolaji *et al.*, 2007).

Researchers believe that some of these plant compounds can accelerate the process of digestion and absorption, and enhance the therapeutic and side effects (Platel *et al.*, 2002; Adams, 2005; Adediji *et al.*, 2008).

In the beginning of the rearing period, fingerlings of Siberian sturgeon were distributed with the same weight in all treatments and control group, but at the

end of two months of rearing, weight increase was observed in treatments with *A. Vera* extract compared to control group ($p<0.05$). The highest weight increase was observed in the treatment fed 1.5% *A. Vera* extract (98.82 \pm 0.21g) and the lowest increase of weight was measured in the control group (62.4 \pm 0.28 g).

Based on studies of Alishahi *et al* (2010) and Alishahi and Abdi (2013), the use of *A. vera* can be effective in the growth of some fish species. The present study showed that the growth indices (WG, SGR, IBW, PER and CF) increased in treatments. The increased growth can be affected by *A. vera* nanoparticles.

By increasing the use of *A. vera* nanoparticles in the treatments, FCR values decreased so that the minimum and maximum amounts of FCR were reported in the 1.5% treatment (0.87 \pm 0.003) and control group (1.28 \pm 0.007), respectively. Furthermore, during the rearing period, no mortality was observed in the

treatments and control groups and survival rate was 100%.

Given that the FCR is one of economic factors of aquaculture, in addition to reducing the cost of feed the consumption of food is also reduced. Also, lower FCR values prevent the pollution of the rearing water (Falahatkar *et al.*, 2007).

So far, different results have been obtained from the effects of *A. vera* on growth of fish species (Farahi *et al.*, 2012). According to Farahi *et al* (2012), food supplements containing *A. vera* were not effective on growth indices (IBW, SGR and FCR) of rainbow trout (*Oncorhynchus mykiss*) in treatments in comparison with the control group. However, in the present study, the positive effects of *A. vera* nanoparticles on the indices have been obtained. Alishahi *et al* (2010) reported that the use of three levels of crude extract of *A. vera* (0.1%, 0.5% and 1%), within 60 days of feeding to *Amphiophus labiatus*, resulted in a significant increase in the amounts of weight gain and FCR. The results of Alishahi *et al* (2010) are in agreement with those of the present study although the recent study showed that 1% and 1.5% *A. vera* extract are more effective.

In another study Farahi *et al* (2012) showed that the use of different levels of *A. vera* (0.1% and 1%) has a positive effect on growth performance in rainbow trout. However, the use of the same level of *A. vera* in Siberian sturgeon had no effect on growth of the fish (Wang *et al.*, 2011). Even though,

in the recent study using the *A. Vera* extract in nanoparticle form had suitable effects on Siberian sturgeon. *A. vera* is formed from 75 potential active compounds such as vitamins, enzymes, minerals, sugars, lignin, saponins, amino acids and salicylic acid (Surjushe *et al.*, 2008).

Improvements on growth performance of fish, can thus be better digestion and absorption of nutrients, as well as better activity of digestive enzymes and intestinal structure (Ngamkala *et al.*, 2010) The improved growth performance in the present study is justified based on the results Namkala *et al.* (2010). So far, no study has been conducted on the effects of nanoparticles of medicinal plants on growth and body composition of aquatic organisms. This study is the first research of this kind, while some studies have been conducted up to now on the effects of medicinal plants on growth of aquatics.

The chemical composition of the body is affected by a combination of diets and amount of daily feeding (Hung *et al.*, 1987; Gawlicka *et al.*, 2002). Results of this study showed no significant differences in protein, lipid, carbohydrate, ash and moisture in Siberian sturgeon carcass in different treatments compared with the control group had no significant difference ($p>0.05$). In other words, body composition was not affected by the use of *A. vera* extract.

With the increased use of *A. vera* extract in the diet, protein content

increased and lipid level was reduced so that the maximum amount of protein and lipid were observed in the 1.5% and 0.5% treatments, respectively, and minimum levels of protein and lipid were observed in the control group. In addition, carbohydrate, ash and moisture content in treatments were associated with a reduction compared to the control.

The results of Mahdavi *et al* (2013) on the effects of adding the essential oil of fennel (*Foeniculum vulgare*) in the diets of juvenile Kutum (*Rutilus kutum*) and the present study conform with each other (except for the amount of body lipid). Zheng *et al* (2009) reported an increase in protein content in the channel catfish (*Ictalurus punctatus*) fed Greek Marjoram (*Origanum heracleoticum*) Shalaby *et al.* (2006) reported that adding a mixture of garlic and chloramphenicol in tilapia diets caused significant increase in protein content and significant reduction in body lipid content. Differences in fish species, the type of diet and the use of different herbal ingredients can cause differences in body composition in fish species.

Using food supplements especially herbal compounds in the form of nanoparticles can lead to more survival and higher absorption in the digestive tract. Some barriers in digestive tract such as mucous can be considered as an obstacle to drug absorption. Some nanoparticles can pass these barriers and improve drug transfer (Ensign *et al.*, 2012). Regarding many

improvements in oral drug consumption, a very wide area among drug delivery systems, more studies are needed in this area (Hughes, 2005).

Considering the results of this research it can be concluded that *A. vera* nanoparticles as a dietary supplement can play a useful role in increasing the growth performance and be used as an additive in diets for Siberian sturgeon.

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