Effect of garlic (*Allium sativum*) and nettle (*Urtica dioica*) on growth performance and hematological parameters of beluga (*Huso huso*)

Z Nobahar[1], H Gholipour-Kanani[1], Sh Kakoolaki[2] and H Jafaryan[1]

[1]Department of Fisheries, Faculty of Agriculture and Natural Resource, Gonabad Kavous University, GonbadKavous, Iran.

[2]Iranian Fisheries Research Organization

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Abstract

In this study, the hematological and growth performance of beluga (*Huso huso*) fed with herbal supplemented diet were studied. Dietary treatments were control (normal diet), 1 g garlic (*Allium sativum*) and 1 g nettle (*Urtica dioica*) per 100g feed for 60 days. No significant difference was observed in growth performance between the groups ($P>0.05$). MCV increased significantly in nettle group compared to control group after 60 days ($P<0.05$). Lymphocyte increased in nettle and garlic groups compared to control group after 20 days ($P<0.05$). In conclusion, it seems that herbal diet can improve hematological parameters and immunocompetent cell activity in this species.

Keyword: growth performance, hematological parameters, garlic, nettle, beluga.

Introduction

The ultimate goal of the aquaculture industry is to optimize growth and to produce high-quality fish. Sturgeons are ancient group of chondrostean fish with fossil records dating back to the lower Jurassic period, which lived in northern hemisphere and are currently highly endangered (Safarpour-Amlashi, Falahatkar, Sattari & Tolouei-Gilani 2011). Beluga (*Huso huso*) is one of the most important species of sturgeon in the Caspian Sea, Black sea and Sea of Azov (Jalali, Hosseini & Imanpour 2010). In recent decades, the economic value, especially because of the production of caviar and meat, lead to increase of sturgeon rearing (Sudagar, Hajibeglou, Jalali, Farahi, Kasiri & Alamshahi 2011).

The use of hematological techniques including evaluation of erythrocytes count, hemoglobin concentration, hemaetocrit and leucocytes count reflecting the health status of fish cultured with all treatments has provided valuable information for fishing biologists in the assessment of fish health (Shalaby, Khattab & AbdelRahman 2006). Garlic (*Allium sativum*), one of the seriously studies herbal plants over the years, has been utilized to fight infections for centuries (Alorainy 2011). Moreover, garlic has the ability of catalase activity in serum and lowering the levels of plasma glucose in fish (Nwabueze 2012). It increases the welfare of fish and can help in the control of pathogens, specially bacteria and fungi. The garlic has several beneficial effects including antioxidant, antihypertensive and antimicrobial properties (Fazlolahzadeh, Keramati, Nazifi, Shirian & Seifi 2011).

Nettle (*Urtica dioica*) belongs to the family Urticaceae, are mostly herbaceous perennial plants and chemical composition shows the presence of acetylcholine, histamine, serotonine and formic acid. It also contains minerals, chlorophyll, amino acid, lecithin, carotenoids, flavonoids, sterols, tannins and vitamins (Krystofova, Adam, Babula, Zehnalek, Beklova, Havel & Kizek 2010). The nettle plant supplement is a common dietary additive for humans and was chosen for its non-toxic chemical composition due to its relatively low cost and availability in northwest of Iran (Safamehr, Mirahmadi

Correspondence Z Nobahar, Department of Fisheries, Faculty of Agriculture and Natural Resource, Gonabad Kavous University, Gonbad Kavous, Iran (email: Zahra.nobahar@yahoo.com)

& Nobakht 2012). The aim of the present study was to evaluate the influence of these two herbal plants on the growth performance and hematological factors of beluga.

Materials and Methods

The study was conducted in Shahid Marjani Sturgeon Fish Propagation and Rearing Center, Gorgan, Iran. A number of 270 fish with mean weight of 30 ± 0.5g randomly selected from holding tank and distributed in 9 fiberglass tanks (three replicates per treatment and 30 fish per tank) filled with 5000L water (flow rate = 100 l/h, temperature = 26.5 ºC, dissolved oxygen = 5.2 and pH = 8.01). Oven dried garlic and nettle were obtained from local supermarket. They were then crushed and mixed with Biomar formulated food (Table1). The experimental diets were supplemented with 1% garlic (group 1), 1% nettle (group 2) and control diet was free from herbal plants. After one-week adaptation, groups were fed diets (5% body weight daily) for a period of 60 days. Fish were hand-fed four times daily (06:00, 12:00, 18:00, and 24:00) according to their satiation.

The parameters of total length, body weight gian (BWI), feed conversion ratio (FCR), specific growth rate (SGR), daily growth rate (DGR) and condition factor (CF) were calculated each 15 days interval according to the below equations.

$WBI\ (\%) = 100 \times (w_f - w_i)/w_i$

FCR (%) = (dry feed intake (g)/wet weight (g)) × 100

$SGR\ (\%\ day^{-1}) = (Ln\ w_f - Ln\ w_i) \times 100/t$

$CF\ (\%) = 100 \times [\text{wet weight (g)}/TL^3\ (\text{cm})]$

$DGR\ (\%) = (w_f - w_i) \times 100/t$

(w_i: initial fish weight, w_f : final fish weight, TL: total length, t: experimental duration in day).

Blood samples of 15 fish from each treatment were collected each 20 days intervals after fish being anesthetized by clove oil extract (25 ppm/l) (GholipourKanani, Mirzargar, Soltani, Ahmadi, Abrishamifar, Bahonar & Yousefi 2011). Haematological parameters including erythrocytes count (RBC), Haemoglobin concentration (Hb), Hematocrit value (Htc), leukocytes count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured using the standard routine techniques (Noga 2010). Differential leukocyte counts were determined using Giemsa staining method of blood smears under a light microscope.

Table 1 Proximity analysis of Biomar formulated food

Protein (%)	Lipid (%)	Fibre (%)	Ash (%)	Moisture (%)
47.2	13.5	4.7	12	10.1

The measured parameters were statistically evaluated using an analysis of variance procedure using one-wey ANOVA, followed by using the Bonferroni. Differences at $p<0.05$ were considered to be significant and all results in the text were stated as mean ± standard error (SE).

Results

Growth parameters

The results of growth parameters are shown in Fig.1-5. No significant difference was observed in growth parameters between trial groups ($P>0.05$). CF increased significantly in fish fed nettle compared with garlic group during first 15 days ($P < 0.05$). No mortalities were observed in the treatment groups and control group during the feeding trial.

Hematological factors

The results of hematological factors are shown in Tables 2 and 3. Hb increased significantly in fish fed nettle compared with garlic and control after both 20 and 40 days of experiment ($P < 0.05$). Htc increased significantly in nettle group compared with control and garlic groups after 60 days ($P < 0.05$). We did not observe any significant difference in MCH between experimental groups ($P > 0.05$). MCHC increased significantly in nettle group compared with control and garlic during40 days post-treatment ($P < 0.05$). Also, garlic treatment showed a significant increase in MCHC compared to control and nettle groups after 60 days post-experiment ($P < 0.05$). MCV decreased significantly in garlic group compared with control and Nettle group after 60 days ($P < 0.05$) post-treatment. However, it increased

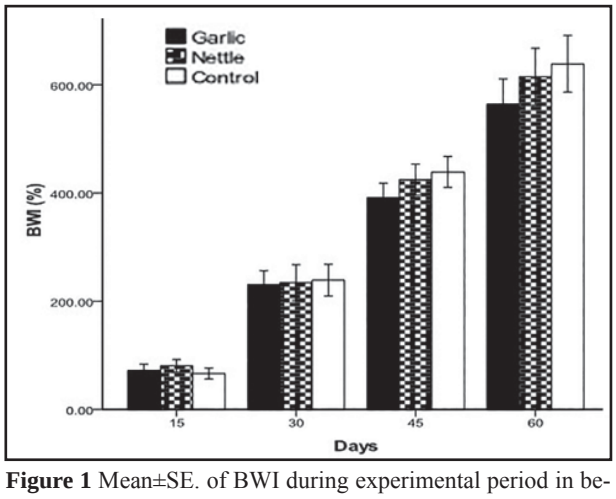

Figure 1 Mean±SE. of BWI during experimental period in beluga, at 26.5 ºC (n=15).

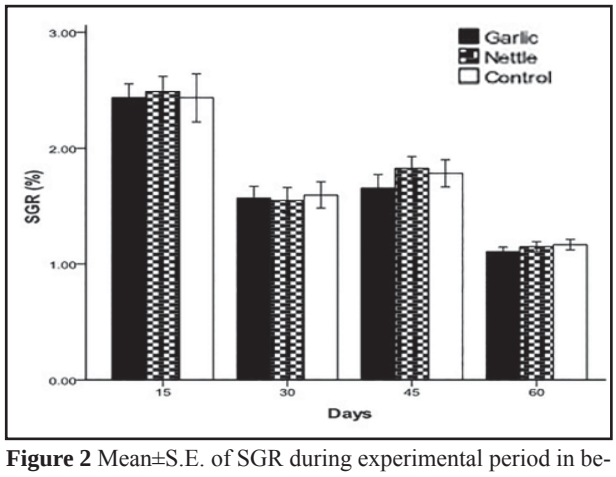

Figure 2 Mean±S.E. of SGR during experimental period in beluga, at 26.5 ºC (n=15).

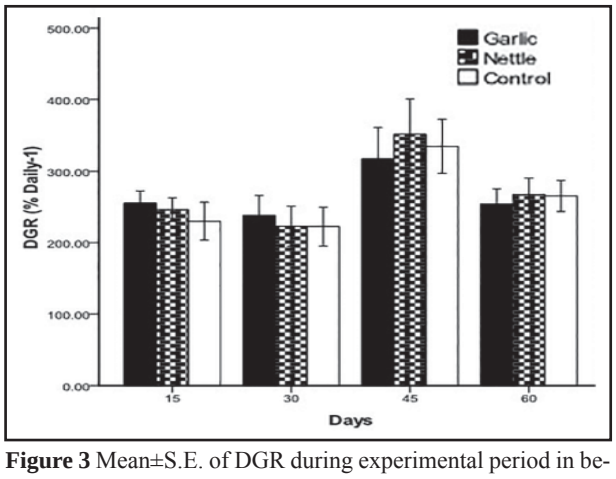

Figure 3 Mean±S.E. of DGR during experimental period in beluga, at 26.5 ºC (n=15).

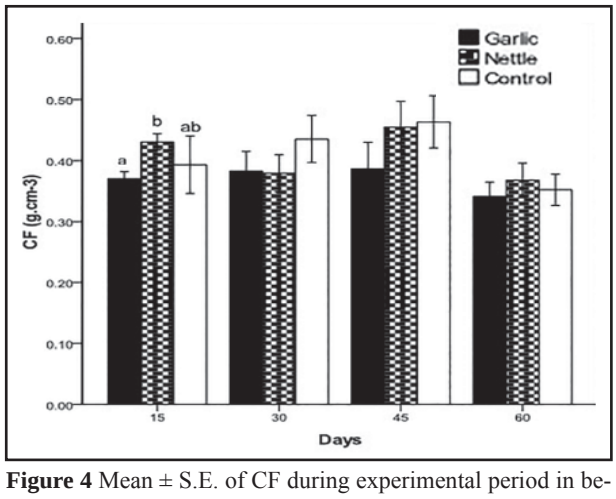

Figure 4 Mean ± S.E. of CF during experimental period in beluga. Different superscript shows significant at P<0.05, at 26.5 ºC (n=15).

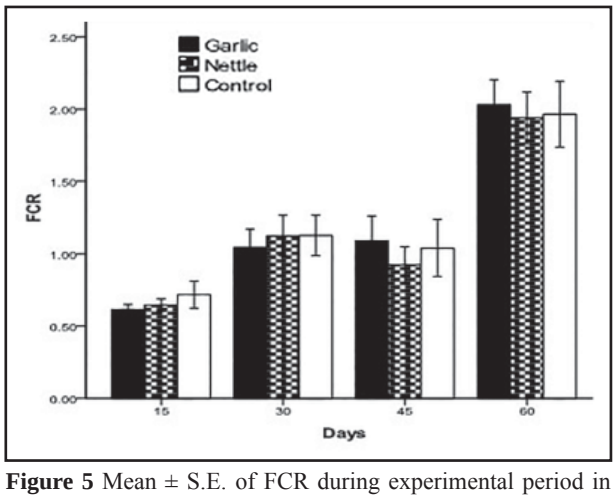

Figure 5 Mean ± S.E. of FCR during experimental period in beluga, at 26.5 ºC (n=15).

significantly in garlic group compared with control and Nettle group after 40 days post-experiment ($P < 0.05$). WBC increased significantly in garlic group compared with control and Nettle group after 40 days post-treatment ($P < 0.05$). Neutrophils had the highest level in nettle group compared to control and garlic after 40 days post-treatment ($P < 0.05$). Lymphocytes increased significantly in nettle group compared with control group after 20 and 40 days post-treatment ($P < 0.05$). Moreover, we observed significant increase in garlic group compared to control in first 20 days ($P < 0.05$). Monocytes showed no significant difference among treatments ($P > 0.05$). Nettle treatment had the highest eosinophil level compared with control and garlic groups on day 60 of the experiment ($P < 0.05$).

Table 2 Hematological factors of beluga fed diets containing herbal plants at 26.5 ºC for 60 days

Treatment	RBC	WBC	Hb	Htc	MCHC	MCH	MCV
20 days after feeding							
Garlic	0.43 ± 0.06 ^{a}	0.28 ± 0.02 ^{a}	3.09 ± 0.25b	26.1 ± 1.67^{a}	12.65 ± 1.44^{b}	123.92 ± 28.89^{a}	982.7 ± 198.25^{a}
Nettle	0.46 ± 0.08 ^{a}	0.27 ± 0.03 ^{a}	7.42 ± 0.52^{a}	23.51 ± 1.76^{a}	34.49 ± 4.00^{a}	306.13 ± 64.03^{a}	874.58 ± 179.83^{a}
Control	0.42 ± 0.08 ^{a}	0.34 ± 0.01^{a}	3.8 ± 0.2b	20.53 ± 1.08^{a}	19.09 ± 1.32^{b}	186.29 ± 39.38^{a}	990.92 ± 211.6^{a}
40 days after feeding							
Garlic	0.6 ± 0.07 ^{a}	0.34 ±0.02^{a}	4.76 ± 0.29^{b}	36.35 ± 2.84^{a}	13.91 ± 1.08^{b}	120.89 ± 26.62^{a}	1017.45 ± 267.06^{a}
Nettle	0.81 ± 0.02 ^{a}	0.16 ± 0.01^{b}	7.26 ± 0.41^{a}	19.23 ± 1.36^{b}	40.47 ± 3.49^{a}	90.43 ± 5.91^{a}	236.29 ± 15.65^{b}
Control	0.68 ± 0.04 ^{a}	0.25 ± 0.02^{b}	5.47 ± 0.29^{b}	26.85 ± 2.82^{ab}	25.83 ± 4.24^{b}	83.59 ± 6.23^{a}	417.8 ± 51.56^{b}
60 days after feeding							
Garlic	0.5 ± 0.04^{a}	0.37 ± 0.02^{a}	6.63 ± 0.48^{a}	13.99 ± 0.69^{b}	47.01 ± 2.03^{a}	146.28 ± 15.74^{a}	308.05 ± 29.01^{b}
Nettle	0.39± 0.03^{a}	0.38 ± 0.03^{a}	6.61 ± 0.6^{a}	28.99 ± 1.56^{a}	22.31 ± 1.27^{c}	204.69 ± 35.29^{a}	872.24 ± 119.73^{a}
Control	0.36 ± 0.05^{a}	0.39 ± 0.03^{a}	5.81 ± 0.23^{a}	17.57 ± 1.00^{b}	34.73 ± 2.6^{b}	245.04 ± 46.85^{a}	753.69 ± 158.19^{a}

Values are mean ± S.E. values with different superscripts in the same day/group and column are significantly different, not sharing common superscript letters are significantly different (P<0.05, n=15).

Table 3 Differential leukocyte counts of bluga fed dietary herbal plant at 26.5 ºCFor 60 days

Treatment	Lymphocyte (%)	Neutrophil (%)	Eosinophil (%)	Monocyte (%)
20 days after feeding				
Nettle	90.00 ± 0.1 ^{b}	6.00 ± 0.2 ^{a}	3.00 ± 1.3^{a}	1.00 ± 0.00^{a}
Garlic	89.93 ± 0.2^{b}	5.4 ± 0.1 ^{a}	3.53 ± 0.2^{a}	1.00 ± 0.00^{a}
Control	89.00 ± 0.00^{a}	7.00 ± 0.00^{b}	3.00 ± 0.00^{a}	1.00 ± 0.00^{a}
40 days after feeding				
Nettle	95.00 ± 0.00^{a}	8.00 ± 0.00 ^{a}	2.00 ± 0.00^{a}	1.00 ± 0.00^{a}
Garlic	90.00 ± 0.1^{b}	7.00 ± 0.1 ^{b}	2.00 ± 0.1^{a}	1.00 ± 0.00^{a}
Control	90.2 ± 0.2 ^{b}	7.00 ± 0.1^{b}	1.8 ± 0.1^{a}	1.00 ± 0.00^{a}
60 days after feeding				
Nettle	90.00 ± 0.2^{a}	7.00 ± 0.2^{a}	2.13 ± 0.09 ^{a}	1.00 ± 0.00^{a}
Garlic	89.8 ± 0.22^{a}	6.5 ± 0.13^{a}	2.6 ± 0.21a^{b}	1.00 ± 0.00^{a}
Control	89.2 ± 0.1^{a}	6.8 ± 0.2^{a}	3.00 ± 0.16^{b}	1.00 ± 0.00^{a}

Values with different superscripts letters in the same day/group and column are significantly different (P< 0.05, mean ± S.E, n=15).

Discussion

The use of immunostimulants is highly recommended in aquaculture and it has become one of routine procedures in fish farming. However, due to its high costs, a large number of these additives are not economical. There are large numbers of plants, which have been used, in traditional medicine that can be replaced as immunostimulant. According to our knowledge, the effects of dietary garlic and nettle on blood parameters of beluga were examined in this survey. In present study, dietary plants showed no significant difference on growth parameters. Ndong & Fall (2007) reported garlic supplemented diet can decrease BWI in juvenile Tilapia. Similar results were obtained by Shalaby *et al*. (2006) in Nile tilapia. Although we observed decrease in BWI in garlic group, this increase was not significant. Thanikachalam, Kasi & Rathinam (2010) reported that the dietary inclusion of garlic peel had no significant effect on weight gain, SGR and FCR of African catfish fingerling, which agrees with our findings. Nwabuez (2012) reported that garlic supplemented diet did not have any significant effect on weight gain of *C. gariepinus* in comparison with control group, but they observed significant increase in final weight in garlic group.

Nettle is a very nutritious food which is easily digestible and is rich in minerals, vitamin C, and pro-vitamin A (Safamehr*et al*. 2012). Viegi, Pieroni,

Guarrera and Vangelisti (2003) reported that nettle could be used in folk veterinary medicine. Awad, Austin & Lyndon (2012) reported that feeding with 1% *Urtica dioica* for 2 months led to highest length and CF compared to the controls in rainbow trout which is in accordance with present study on the other hand final weight, weight gain and SGR of treatments were significantly higher than the control which disagree with present study. In addition, Dugenci, Arda & Candan (2003) studied the influence of nettle on Rainbow trout; they observed no significant difference in growth parameters that is in agreement with present study. Nettle was used in broiler; Safamehr *et al*. (2012) reported that nettle could be used as growth promoters in broiler diets. Hosseinimansoub (2011) reported significant increase of FCR and weight body in broiler fed with nettle.

Employment of hematological techniques is valuable source for fish biologists in health assessment and reflects the health status of fish cultured with all treatments (Shalaby *et al*. 2006). The primary function of RBC in fish is to transport oxygen from the gills to the various tissues of the body, an action carried out by the haemoglobin found in RBC. Shalaby *et al*. (2006) reported significant increases in RBC, Hb and Htc of Nile tilapia fed with garlic that is similar to the results of this study. It is assumed that the increase of blood indices may be attributable to a defense reaction against garlic, which occurs by stimulation of erythropoiesis (Fazlolahzadeh *et al*. 2011). Faisal (2003) reported significant decrease of MCH and MCV in Cat fish (*Clarias garepins*) fed with garlic that is also similar to our findings in the present study. Garlic increases super oxide dismutase (SOD) activity in blood that is known as non-specific defense system in *Oreochromis niloticus* (Nwabuez 2012). Leucocytes play an important role in non-specific or innate immunity and their count can be considered as indicator of the health status of fish (Fazlolahzadeh *et al*. 2011). Dugenci*et al*. (2003) observed that nettle improved immunity in Rainbow trout. Garlic quickens macrophage phagocytosis, a process by which microorganisms and cellular debris is engulfed and destroyed (Lau, Yamasaki & Gridley 1991).

To conclude, garlic and nettle have influenced some blood parameters and can enhance some innate immune parameters in beluga. Further research is required to clarify the action mechanisms of garlic and nettle as well as the appropriate inclusion dose and feeding period in great sturgeon.

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اثرات سیر و گزنه بر عملکرد رشد و پارامترهای خونی فیل ماهی (*Huso huso*)

زهرا نوبهار[1*]، حسنا قلی پور کنعانی[1]، شاپور کاکولکی[2]، حجت الله جعفریان[1]

۱ گروه شیلات، دانشکده کشاورزی و منابع طبیعی، دانشگاه گنبدکاووس، گنبد کاووس،ایران

۲ سازمان شیلات ایران، ایران

چکیده

در این مطالعه، پارامترهای خونی و رشد فیل ماهیان(*Huso huso*) تغذیه شده با دو مکمل گیاهی مورد بررسی قرار گرفت. تیمارها به صورت، تیمار شاهد (بدون مکمل گیاهی)، تیمار ۱ گرم سیر در هرکیلوگرم غذا و تیمار۱ گرم گزنه در هرکیلوگرم غذا برای ۶۰ روز فراهم شدند. در پایان دوره ۶۰ روزه هیچ اختلاف معنی‌داری در پارامترهای رشد بین گروه‌ها مشاهده نشد ($p>0/05$). همچنین بعد از دوره ۶۰ روزه شاخص MCV در تیمار گزنه افزایش معنی‌داری نسبت به گروه کنترل نشان داد ($p<0/05$). افزایش معنی دار در تعداد لمفوسیت‌ها در گروه سیر و گزنه نسبت به گروه کنترل بعداز ۲۰ روزمشاهده گردید ($p<0/05$). بطور کلی، به نظر می‌رسد که جیره‌های گیاهی می‌توانند برخی پارامترهای هماتولوژی و ایمنولوژی را در این گونه بهبود دهند.

واژه‌های کلیدی: پارامترهای رشد، پارامترهای هماتولوژی، سیر، گزنه، فیل ماهی.

*نویسنده مسئول: Zahra.nobahar@yahoo.com