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Comparative Effect of Raw Fiber (Vitacel) and Alginic acid (Ergosan) on Growth Performance, Immunocompetent Cell Population and Plasma Lysozyme Content of Giant Sturgeon (*Huso huso*)

M. Heidarieh[1,*], M. Soltani[2], A.H. Tamimi[3], M.H. Toluei[4]
[1] Agricultural, Medical and Industrial Research School (AMIRS-NSTRI), Karaj, Iran.
[2] Aquatic Animal Health Departments, Faculty of Veterinary Medicine, University of Tehran, Iran.
[3] University of Applied Science and Technology, Tehran, Iran.
[4] Aquaculture section, Iran Fisheries, Bandare-Anzali, Gilan Province, Iran.

* Corresponding Author: Tel.: +98.9124957009; Fax: +98.2614464061;
E-mail: mheidarieh@nrcam.org

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Abstract

The imunnocompetent cell population size including lymphocyte, neutrophil and eosinophil populations, lysozyme activity in plasma and the growth performance were assessed in great sturgeon *Huso huso* after oral administration of Ergosan at 0.5% of the feed and Vitacel at 1.3% of the feed for 90 days. Sampling was performed on 15 and 90 days during feeding trial. Generally, a significant increase was seen in the lymphocyte count of fish received Ergosan compared to both Vitacel and control groups 90 days post-treatment ($P<0.05$). Interstingly, at the Neutrophil profile of fish fed Vitacel and Vitacel-Ergosan combination was observed significant increase on 90 days after trial ($P<0.05$). The number of eosinophils tended to be higher in Vitacel group ($P<0.05$) on day 90 and was significantly higher ($P<0.05$) than in Ergosan and control. Also, lysozyme activity in plasma of treatment fish by Ergosan and Vitacel was significantly higher than control group on 15 and 90 days post-treatment ($P<0.05$). In addition, the weight of fish fed diet containing Ergosan and/or Vitacel had a significant increase compared to control group on days 15 and 90 of trial ($P<0.05$).

Keywords: Ergosan, Vitacel, *Huso huso*, Lysozyme, Growth performance.

Gelişkin Mersin Balıklarının (*Huso huso*) Büyüme Performansına, Hücre Popülasyonunun Bağışıklık Yetisine ve Plazma Lizozom İçeriğine Ham Selüloz (Vitacel) ve Alginik Asit (Ergosan)'ın Karşılaştırmalı Etkileri

Özet

Yetişkin mersin balıklarının (*Huso huso*) 90 gün boyunca yemine %0,5 oranında Ergosan ve %1,3 oranında Vitacel'in katılarak beslenmesi sonrası lenfosit, nötrofil ve eozinofil populasyonlarını da içeren hücre sayıları; bağışıklık yapısı, plazmadaki lizozom aktivitesi ve büyüme performansı değerlendirilmiştir. Besleme denemesi boyunca 15. ve 90. günde örnekleme yapılmıştır. Genel olarak, 90 günlük yemleme sonrası Ergosan alan balıklardaki lenfosit sayısı hem Vitacel alan balıklara hem de kontrol grubuna kıyasla önemli derecede ($P<0,05$) yüksek çıkmıştır. Şaşırtıcı şekilde, 90 günlük deneme sonrasında Vitacel ve Vitacel-Ergosan kombinasyonu ile beslenen balıkların nötrofil değerlerinde önemli derecede ($P<0,05$) artış gözlemlenmiştir. Eozinofil sayısı 90. günde Vitacel ile beslenen grupta önemli derecede ($P<0,05$) yüksek çıkma eğilimi göstermiştir ve Ergosan ile beslenen gruba ve kontrol grubuna kıyasla yine önemli derecede ($P<0,05$) yüksek çıkmıştır. Plazmadaki lizozom aktivitesi de Ergosan ve Vitacel muamelesi gören balıklarda yemleme sonrası 15 ve 90. günlerde kontrol grubundan önemli derecede ($P<0,05$) yüksek çıkmıştır. Buna ilaveten, denemenin 15 ve 90. günlerinde Ergosan ve/veya Vitacel ile beslenen balıkların ağırlıklarında kontrol grubuna kıyasla önemli derecede ($P<0,05$) artış olmuştur.

Anahtar Kelimeler: Ergosan, vitacel, *Huso huso*, lizozom, büyüme performansı.

Introduction

The non-starch polysaccharides (NSP) are normally used in fish feed as the fiber. This compound has significant effects on digestion and metabolism of nutrients in poultry and pigs (Johnston *et al.*, 2003). Dietary fiber may affect animal health through several possible mechanisms, including hypertrophy of the gastrointestinal tract, the proliferation of epithelial cells of jejunum and ileum, increased secretion of Musina ileum. These functions may cause a change in the microbial population and improving the digestibility of food consumed (Johnston *et al*., 2003).

Most sources of fiber used in animal feed are by products of food manufacture built (Backers, 2007). According to the original material, the crude fiber content of these products varies greatly and ranges

from 10 to 35%. They may also include some unnecessary substances such as mycotoxin that may affect the animal health requirements (Backers, 2007). Vitacel is a pure substance with at least 70% of crude fiber (Backers, 2007). This substance has been marketed in Europe as Vitacel R 200 and has demonstrated an ability to stimulate the digestibility of nutrients (Backers, 2007). For example, studies have been conducted at the University of Mexico to examine the effect of crude fiber on the increase in intestinal villi. The majority of results have been taken in the duodenum, the initial part of the intestine where most digestive enzymes are produced. This survey showed that not only clears but also tendencity of intestinal villi is significantly longer in group Vitacel. Much of the finest fibers stimulate circulation of blood in the intestinal villi, which improves the digestability of nutrients (Backers, 2007). Besides mammals, there is no data available on the effects of Vitacel on aquatic animals, particularly fish. The immunomodulatory effects of glucans were found to be different as regards the species of fish, the manner of administration and doses (Robertsen *et al*., 1990). Alginic acid (Ergosan) improve of the head kidney phagocyte functions through their movements within the site of injection of alginate in certain fish species by increasing their production of chemotactic factors and their function phaogocytosis (Dalmo and Seljelid, 1995; Oh and Choi, 1998). In a study by Peddie *et al*. (2002) It has been shown that the mechanism of alginates on the fish immune system may improve the oxygen transfer through the cell membrane of lymphocytes and macrophages, raising metabolic activity, which results in improved disease resistance and increased capacity for renovation of injured tissues (Montero-Rocha *et al*., 2006).

Despite numerous studies on the effects of Ergosan in aquatic animals, but there is no data available on the effects of Vitacel alone or in combination with Ergosan in aquatic animals. Therefore, the aim of this study was to evaluate the effects of Vitacel alone or in combination Vitacel-Ergosan on growth performance and some immune variable of the giant sturgeon (*Huso huso*).

Materials and Methods

Fish

Juveniles sturgeon *(Huso huso)* weighing 100-110 g from a Caspian Sea fish farm were used for the experiments. The fish were acclimated to new conditions provided in 12 fiberglass tanks with aeration for 10 days. Fish were fed at 1.5% body weights 3-4 times daily with commercial pelleted diet (BioMar, France) used for rainbow trout. The feed was handed-fed to the fish, Prior to the experiment fish were checked for any abnormality and mortality for 10 days. Water quality parameters including water temperature, pH and dissolved oxygen were 22-25°C, 7-7.4, and 5-6 mg/L respectively. Fish were randomly divided in 4 groups, 47 each group in three replicates. First group was fed using 0.5% Ergosan per kg food for 90 days. Second group was fed 1.3% Vitacel per kg food for 90 days. Third group was fed using a combination of Ergosan at 0.5% and Vitacel at 1.3% per kg food for 90 days. Fourth group was considered as control using normal food.

Blood Sample Collection and Lysozyme Assay

Blood samples were collected from the caudal vein of fish after being anesthetized with clove oil at 100 mg/L on 15 and 90 days post-trial. Blood samples were centrifuged at 7000×g for 30 minutes and separated sera were used for lysozyme assay described by Ellis (1990) with some modifications. Briefly, aliquots (1.75 ml) of *Micrococcus lysodeikticus* suspension (0.375 mg/L of 0.05 sodium phosphate buffer, PH 6.2) was mixed with 250 µl of each sample and the optical density was measured after 15 and 180 seconds by spectrophotometer at 670 nm. PBS was used as die blank and results were expressed in amount of lysozyme (µg) per one milliliter of plasma sample. At the same time of collection, the blood smears were obtained, air dried, fixed in 96% ethanol for 30 min then stained by Giemsa staining for 30 min. The stained smears were then examined for leucocyte differential count under light microscope (Khoshbavar-Rostami *et al*., 2006).

Biometry and Growth Performance

The total weight of treated fish was measured every 10 interval days using all experimental fish.

Statistical Analysis

The obtained data were statistically analyzed using SPSS and Excel software and one way ANOVA and significant difference was defined at $P<0.05$ as confidence.

Results

Lysozyme Content

Results of lysozyme contents are shown in Figure 1 and 2. Interestingly, a significant increase was obtained in groups of Viatcel and/or Ergosan day 15 and 90 of trial at optimal doses of immunostimulant agents compared to the control group ($P<0.05$) (Figure 1 and 2).

However, no significant effect on lysozyme contents in plasma was observed after day 15 and 90 of trial at optimal doses of Vitacel and/or Ergosan ($P<0.05$) (Figure 1 and 2).

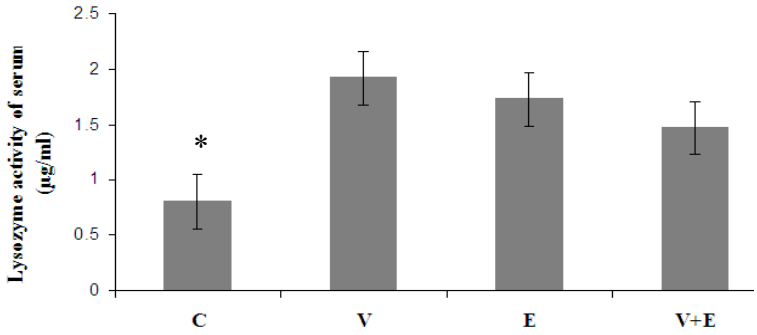

Figure 1. The level of lysozyme in fish plasma of *Huso huso* following oral administration Ergosan and/or Vitacel 15 days - post treatment at 22°C (Mean ± S.D).
C: control group, V: Vitacel group, E: Ergosan group, V+E: Ergosan-Vitacel group, * Values are significantly different (P<0.05).

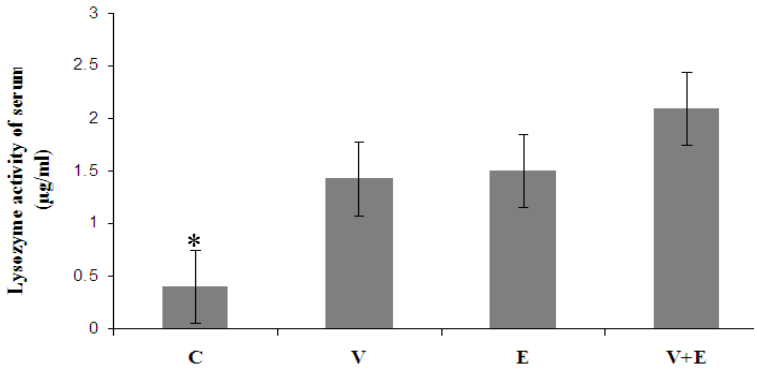

Figure 2. The level of lysozyme in fish plasma of *Huso huso* following oral adimmsrration Ergosan and/or Vitacel 90 days - post treatment at 2 2 ° C (Mean ± S.D).
C: control group, V: Vitacel group, E: Ergosan group, V+E: Ergosan-Vitacel group, * Values are significantly different (P<0.05).

Differential Count

The lecucytic profile of the experimental fish following oral administration Ergosan and/or Vitacel are shown in Table 1. Significant difference was seen among the counts of Lymphocyte, neutrophil and eosinophil population in Ergosan, Vitacel and Vitacel-Ergosan combination groups at the end of trial (P<0.05). Interestingly, lymphocyte count in Ergosan group was significantly higher than the other groups on day 90 of expriment (P<0.05). Also, neutrophil counts in Ergosan group was significantly decreased at the end of experiment compared to both Vitacel and Vitacel-Ergosan groups (P<0.05). At the Neutrophil profile of fish fed Vitacel-Ergosan was seen significant increase on 90 days after trial (P<0.05).

Hence, in Ergosan group was showed a significant decrease in Eosinophil count at the end of the trial compared to the other groups (P<0.05). The number of eosinophils tended to be higher in Vitacel group (P<0.05) on day 90 and was significantly higher (P<0.05) than in Ergosan and control.

Comparison of the lecucytic profile of the experimental fish obtained two agents (Vitacel and/or Ergosan) indicated Ergosan treatment induced a higher count of lymphocyte and lower count of eosinophil proliferation compared to Vitacel at day 90 of Trial (P<0.05). In contrast, the Vitacel-Ergosan combination and Vitacel gave a significantly higher neutrophil counts compared to Ergosan alone and control groups on day 90 (P<0.05).

Growth Performance

The results of growth are shown in Fig. 3. The mean weight of fish fed diet contained Ergosan, Vitacel and Ergosan-Vitacel was significantly increased compared to control group on 15 and 90 days post-treatment (P<0.05) (Figure 3). However, no significant differences were observed in weight increase among groups of Ergosan, Vitacel and a combination of Ergosan-Vitacel (P<0.05). Additionally, the progress of weight increase showed that feeding fish with Vitacel, Ergosan and a combination of Vitacel-Ergosan did not cause any increase in the weight of fish until 4 weeks post-feeding (Figure 3).

Discussion

No mortality occurred during the 90-day test of the effect of Ergosan and/or Vitacel on certain biological variables and immunological juvenile sturgeon weighing approximately 100 g in all

Table 1. Leucocyte Profiles of *Huso huso* Following Oral Administration Ergosan and/or Vitacel at 22°C

Lymphocyte (%)	Time post-treatment (day)	
	15	90
A	44[b]	52[d]
B	43.33[b]	24.33[b]
C	41.33[a]	23[a]
D	53.67[c]	42[c]
Neutrophil (%)		
A	39.33[d]	32[a]
B	32.67[b]	46.67[b]
C	35.67[c]	49.33[c]
D	22[a]	32[a]
Monocyte (%)		
A	1.33[a]	1.30[a]
B	1.00[a]	0.80[a]
C	1.33[a]	1.20[a]
D	1.00[a]	1.00[a]
Eosinophil (%)		
A	17[a]	14.7[a]
B	22.33[b]	27.66[c]
C	21.66[b]	25.33[b]
D	24.33[c]	25.66[b]

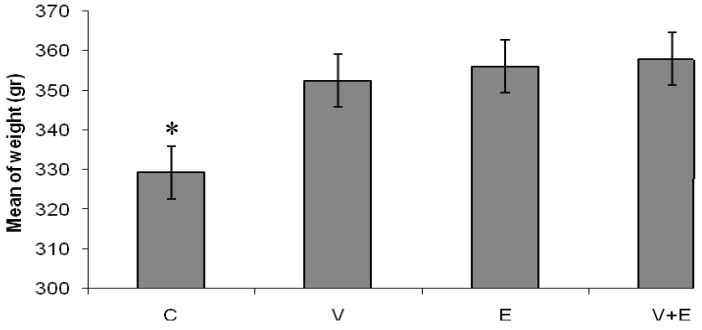

Figure 3. Mean of weight of *Huso huso* following oral administration Ergosan and/or Vitacel 90 days post-treatment at 22°C (Mean ± S.D). C: control group, V: Vitacel group, E: Ergosan group, V+E: Ergosan-Vitacel group, * Values are significantly different ($P<0.05$).

experimental groups. Based on average weight obtained in this study for tested fish, Vitacel and Ergosan application as a food additive may be considered as enhancers of growth.

The effect of the Vitacel and Ergosan on lysozyme production in plasma was studied to find out whether raw fiber such as Vitacel also could stimulate lysozyme production.

Interestingly, the present work demonstrates that both vitacel and Ergosan stimulate lysozyme production in plasma and due to improve immune system of sturgeon.

Based on previous studies, higher vertebrate lysozyme is in polymorphonuclear and mononuclear phagocytes, and Paneth cells which are generalized intestinal epithelial cells (Cohn and Wiener, 1963; Dohrman *et al*., 1994; Paulsen *et al*., 2003). Also, the presence of lysozyme activity in plasma, skin mucus, organs and fish egg has been well documented in different fish species (Engstad *et al*., 1992; Fletcher and White, 1973; Yousif *et al*., 1994; Paulsen *et al*., 2003)

The increase of lysozyme contents by an elevation in lysozyme mRNA suggests that the stimulated lysozyme secretion is started by enhanced lysozyme gene transcription (Paulsen *et al*., 2003). Immunstimulant agents such as β-glucan and LPS stimulated increase in lysozyme activity and gene transcript in Atlantic salmon macrophages (Paulsen *et al*., 2003). Furthermore, it is well established that higher vertebrate lysozyme production in macrophages is an indicative of lysozyme gene activation of the during macrophages maturation (Cross *et al*., 1988). These may have been responsible for higher lysozyme contents in Vitacel and/or Ergosan than control group.

On the other hand, LPS compound for instance Ergosan are known stimulators of cytokines like IL-1

and TNFα in mammalian, salmonid macrophages (Robertson *et al*., 1994; Jang *et al*., 1995; Paulsen *et al*., 2003) and in rainbow trout (Peddie *et al*., 2002), That TNFα, an inflammatory cytokine which is also produced almost solely by macrophage, may hypothetically participate in autocrine stimulation of lysozyme production (Paulsen *et al*., 2003) and so, as neutrophils contain mainly preformed lysozyme in their granules while mature macrophage retain the ability to synthesize new protein, lysozyme mRNA levels in vivo specifically monitor changes in macrophage biosynthetic and secretory activity stimulate by phagocytic and T cell derived stimuli (Paulsen *et al*., 2003).

In another studies were demonstrated that LPS compound induces polyclonal proliferation of salmonid lymphocytes (Warr *et al*., 1983; Paulsen *et al*., 2003), respiratory burst and phagocytic activity of macrophages in fish (Paulsen *et al*., 2003). Consequently, comparison of these data on the concentration of plasma lysozyme in fish has shown that the level of lysozyme is dependent on various factors. This result is supported by changing the appearance of neutrophils and lymphocytes in fish fed with Ergosan and/or Vitacel as immunestimulant substance.

Morever, increased weight of fish fed vitacel and Ergosan is an indicative of increased levels of plasma growth hormone (Saurabh and Sahoo, 2008) in which it has positive effect on the excretion of lysozyme content in plasma of fish.

A substantially, higher lysozyme production was observed by Vitacel and Ergosan compared to the control group may not be due to differences in the functional organization of fish, but it could due to the proportion of cells in particular organs involved in neutralization of the Vitacel and Ergosan.

Accordingly, previous investigations have been conducted the use of Ergosan in vaccine formulations has given very good antibody responses because it seem to stimulate lymphocyte proliferation (Heidarieh *et al*., 2010; Faghani *et al*., 2008; Montero-Rocha *et al*., 2006).

Eosinophils are contributed in the defense against parasitic infections, in the regulation of hypersensitivity reactions, and in the destruction of cancer cells (Pitol *et al*., 2007; Shadkhast *et al*., 2010). The eosinophil counts of Vitacel (raw fiber) group exhibited significant increasing, as seen by Kaufhold *et al*. (2000). Based on Kaufhold *et al.* (2000) report, up till now, there is no data on raw fiber effects on eosinophil counts (Kaufhold *et al*., 2000). Also, this may have contributed to the enhancement of immunoglobulin E production in fish fed Vitacel compared to the other groups (Kaufhold *et al*., 2000).

Thus, the results of this study elucidate the enhanced lysozyme production in plasma and growth performance reflects application of vitacel and Ergosan in that *H. huso* juvenile.

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