

## THE USAGE OF COTTONSEED MEAL INCLUDING DIFFERENT AMOUNTS OF GOSSYPOL IN DIET OF RAINBOW TROUT (*ONCHORHYNCHUS MYKISS*)

SIAMAK YOUSEFI\*, MONA IZADIAN\*\*

\* *Department of Biology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran. (Administrator & Editor) siamak\_yousefi1@yahoo.com*

\*\* *Shilamayesh consulting engineers co. P.O.Box: 15175-583 Tehran, Iran. Corresponding author: monaizadian@yahoo.com*

### UPOTREBA BRAŠNA OD SEMENA PAMUKA SA RAZLIČITIM SADRŽAJEM GOSIPOLA U ISHRANI KALIFORNIJSKE PASTRMKE (*ONCHORHYNCHUS MYKISS*)

#### *Abstract*

Cotton culture is common in many of area of Iran, especially Khorasan province. This study is considering the usage of cotton seed meal with different amount of Gossypol in rainbow trout (*Onchorhynchus mykiss*) diet.

In this examination, the effect of different amounts of Gossypol (common cotton seed, cotton seed with low Gossypol, and cotton seed without Gossypol in Rainbow trout growth in (GFT<sup>2</sup>) stage was examined. At the beginning of the experiment, the fish's weights were 140 gr. In this experiment, 240 Rainbow trout were kept in the training ponds with the 2.240×1.10 meter square dimensions and 0.75 meter depth. Each treatment consisted of 20 Rainbow trout fish. The required water for culturing was provided from a spring in a research center (near Tehran) which was close to the farm with mean temperatures of 14 Celsius.

We consider out four experimental diets including common cotton seed, cotton seed with low Gossypol, and cotton seed without Gossypol for feeding during a six week cultural period.

The diets which consisted of diverse levels of common cotton seed had significant differences ( $\alpha=0.5\%$ ) on weight increasing, total length increasing, standard length increasing and conversion factor. In comparing between different treatments, fish fed with the diet which had common cotton seed had significant differences in the statically sight in weight, conversion factor, FER, total and standard length regarding the other diets and also had the worst condition.

**Key words:** *cotton seed meal, Gossypol, nutrition, rainbow trout, (Onchorhynchus mykiss)*

## INTRODUCTION

Due to the increase in human population and the need for animal protein, especially white meat, fish has found its real position in the everyday diet of human beings. There is a lot of room for growth and profitability and using new alternative materials seems necessary. One of the materials which can be substituted for some of the imported and expensive food items is cottonseed meal. Cotton production in Iran, about 352 thousand tons is estimated that 37/97 percent of the irrigated cotton farms are derived. Annual production of seed cotton is more than 2 million tons (Iranian Agriculture Ministry, 2004). Production efficiency of irrigated and rained cotton farms in Iran has been alternative 2566 kg/hac & 1403 kg/hac (Samavat.2000).

Although different varieties of cotton have cultured without Gossypol, but lower yield per hectare means that they are not used largely in agriculture. So most of cottonseeds cultivated in the world still have a noticeable amount of Gossypol. Smith et al. (1988) reported that rainbow trout fish which was fed with plant or animal protein were not significantly different in growth, carcass composition and taste. Morales et al. (1994) reported that partial replacement of fishmeal (40%) by cotton seed meal had a good effect on growth, feed conversion ratio and energy utilization of rainbow trout. Dabrowski et al. (2000) reported that fishmeal replacement by solvent-extracted cottonseed meal had good results on reproductive and growth of adult rainbow trout fish. Dabrowski (2001) also reported that rainbow trout utilized cottonseed protein very efficiently and up to 75% and 25% of fishmeal protein can be safely replaced by cottonseed meal in diets for adult and juveniles rainbow trout, respectively. Lee et al. (2002) reported that rainbow trout fish can absorb proximately 35-50% of dietary Gossypol, and most of the absorbed Gossypol seemed to be excreted.

Lee et al., (2002) also reported that fishmeal could be entirely replaced by a mixture of plant proteins and animal by-product proteins. Their findings suggest that Cottonseed meal can be used as a good protein source at least 15% in juvenile rainbow trout diets. Lee et al. (2002) further reported that high levels of cottonseed meal had bad effects on the tissues of mature rainbow trout fish. Cheng and Hardy (2002) reported that cottonseed meal could be used at the inclusion rate of 10% in rainbow trout feed formulations. Lee et al. (2006) reported that in rainbow trout juveniles, cottonseed meal could be used as a plant protein source at the level of at least 15% of dietary component (25% of fishmeal protein replacement) without detrimental effects on fish growth. Rincharde et al. (2003) reported that cottonseed meal, which replaced 50% of fishmeal in diets, caused good growth in rainbow trout brood stocks.

## MATERIALS AND METHODS

The experiments were carried out from April until May of 2009. The study was conducted at the Research Center of Agriculture and Natural Resources of Tehran, 13 km away from Tehran (Capital of Iran). Twelve channels (2.240 m long, 1.10 m wide and 75 m deep) supplied with flow through spring water (temperature 14°C) were used. For experiments, 240 rainbow trout (with initial mean body weight of  $140 \pm 5$  g) were

transferred to 12 channels (20 in each) and fed with a commercial rainbow trout diet for an adaptation period of two weeks.

A one factor Completely Randomized Design (CRD) experiment was conducted for this experiment. Four treatments (diets) were tested; Diet -1(Control), Diet -2(with cottonseed meal and without Gossypol), Diet-3(with cottonseed meal and low Gossypol), Diet-4(with cottonseed meal and Gossypol). All treatments had three replicates. Experimental diets were set by the software, "U.F.F.D" and under tables (1994) NRC. The cottonseed meal diets were used in addition to other materials such as flour, corn flour, soybean meal, corn gluten, fish powder, soybean oil, vitamin and mineral supplements, soybean oil, vitamin and mineral supplements. Certain amounts of molasses were used for more consistency in the food pellets of different diets, amount of energy, protein, and etc. Diets were not significantly different from each other. Indicates measured in this study were: weight gain, feed conversion rate, body length, carcass quality (at the end were evaluated to determine the carcass quality of 5 fish of each replication) and percentage loss (It is worth mentioning that there were no casualties during the period.)

All the diets were pelletized using a meat grinder and dried using a laboratory oven-drier at 50 °C for 24 hours. The fish were fed with the test diets three times a day for 6 weeks. Proximate analyses were carried out for both ingredients and diets.

Fish were bulk weighted using a digital balance and counted fortnightly. Data on mortality, growth, and feed utilization were collected accordingly.

All statistical analyses were performed using SPSS. In order to compare the results of the statistical test with that of conventional ANOVA, one-way analysis of variance was performed. Duncan's multiple range (Duncan, 1955) test for means and LSD test to identify the significance of difference between any pair of treatment means were used. All differences were regarded as significant at  $p < 0.01$ .

## RESULTS

ANOVA shows that weight gain effect on weight gain in repeating the test levels not significant. However, between different treatments and also between different times according to test F, weight gain difference is highly significant ( $\alpha=1\%$ ). ANOVA showed that the combined effects of time and treatment on weight gain is highly significant ( $\alpha=1\%$ ). The combined effects of time and frequency levels on weight gain in the test is significant ( $\alpha=5\%$ ). Comparison of different treatments indicated that the two treatments in terms of early SNK grouping has been weight gain. Control treatments (treatment A) treatments, respectively, three and four treatment groups are compared next ( $\alpha=5\%$ ). It is noteworthy that the 1% level, two remain at the forefront of treatment SNK grouping in terms of weight gain has been treated and the control group is still but one and three treatments in a similar group (Group III) located Are ( $\alpha=1\%$ ).

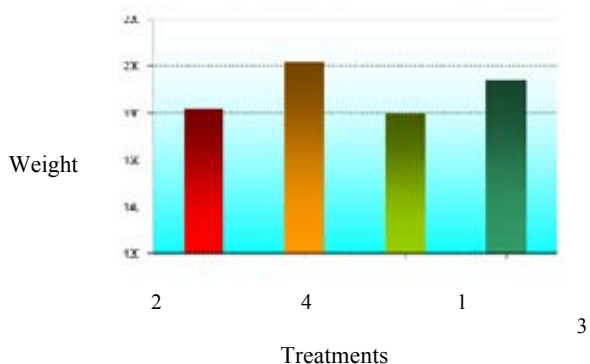
The effect of time (weeks) shows that week listed in terms of weight gain to be divided into six distinct groups. So that weight gain in six weeks, which reached its maximum more than a week, has been a significant increase. Fifth week, the fourth, third, second and first, are followed by ( $\alpha=5\%$ ). The effect of time (weeks) on weight gain in the 1% probability level is also a similar situation ( $\alpha=1\%$ ). ANOVA showed that among treatments and also between different times according to test F, the difference between conversion factors is highly significant ( $\alpha=1\%$ ). Such a feature can be combined in time and treatment effect on the conversion ratio in the test levels to be quite significant ( $\alpha=1\%$ ).

Comparison of the mean is concluded, the two treatments at 5 percent ( $\alpha=5\%$ ) at the top category SNK, located in terms of conversion ratio compared with the treatments that are not desirable. Tuesday and one treatment, a treatment to a significant reduction in the level of 5 percent ( $\alpha=5\%$ ) and the second group are in better condition than the ones treated four of his show. In the last four treatment groups are compared to other treatments that better feed efficiency shows ( $\alpha=5\%$ ). It also can be in a situation likely to be seen as a percent ( $\alpha=1\%$ ). The effect of time (weeks) shows that week listed in terms of conversion ratio of six distinct groups are divided. So that FCR in the sixth week to the minimum value reached more than a week has decreased significantly. Fifth week, fourth, second, third and first, respectively, are followed by ( $\alpha=5\%$ ). The effect of time (weeks) on the conversion ratio at 1% probability level is also a similar situation ( $\alpha=1\%$ ).

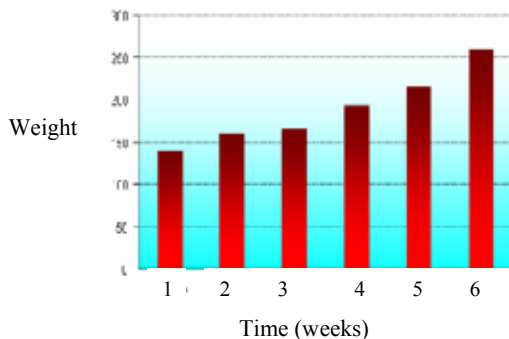
Conclusion of the ANOVA is repeated on the entire length of the test levels are not significant. However, between different treatments and also between different times according to test F, the total length difference is highly significant ( $\alpha=1\%$ ). Such a feature can be combined in time and treatment effects on total length can be observed. However, the combined effect of time and repeated throughout the entire level on the test is not significant. Comparison of different treatments indicates that both treatment and control treatment (a treatment) SNK grouping at the top in terms of the total length have between them there is no significant difference ( $\alpha=5\%$ ). Treatments three and four, in subsequent groups are located in this comparison ( $\alpha=5\%$ ). At 1% probability level, treatments one, two and three are located in the top grouping in terms of total length SNK, but treatment four isolated in the next group ( $\alpha=1\%$ ).

The effect of time (weeks) shows that the first weeks to sixth in terms of total length to be divided into six distinct groups. So that the total increase during the sixth week, which reached its maximum more than a week has been a significant increase. Fifth week, the fourth, third, second and first, are followed by ( $\alpha=5\%$ ). The effect of time (weeks) over the entire length of the 1% probability level is also a similar situation ( $\alpha=1\%$ ).

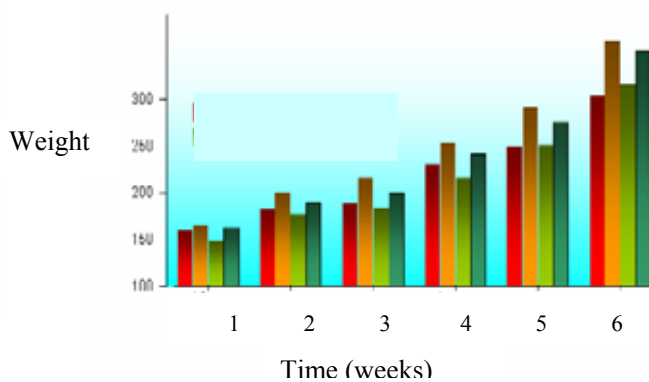
Analysis of variance concluded that the effect of repeated attributes, such as before, on the length of the standard test levels are not significant. However, between different treatments and also between different times according to test F, standard length difference is highly significant ( $\alpha=1\%$ ). Such a feature can be seen in the combined effect of time and treatment effects on standard length. Combined effects of time and repetition, but on the whole length of the test levels are not significantly observed. ANOVA showed that both treatment and control treatment in an error of 5 percent ( $\alpha=5\%$ ) at the top category SNK, in terms of standard length are located. Treatment three, compared to treatment with a significant reduction in the level of 5 percent ( $\alpha=5\%$ ) and four treatments with a significant reduction in both levels have been tested. However, in testing a percent ( $\alpha=1\%$ ), treatments 2 and 3 and the control treatment (a) in the top group and statistically significant differences do not show. However, the four treatments with other treatments are statistically different. ( $\alpha=1\%$  and  $5\%$ ). Effect of time (weeks) suggests that such attributes as the previous standard length during the first weeks until the sixth is divided into six distinct groups. So that the increase in standard length in the sixth week, which reached its maximum more than a week has been a significant increase. Fifth week, the fourth, third, second and first, are followed by ( $\alpha=5\%$ ). The effect of time (weeks) of standard length in 1% probability level is also a similar situation ( $\alpha=1\%$ ).



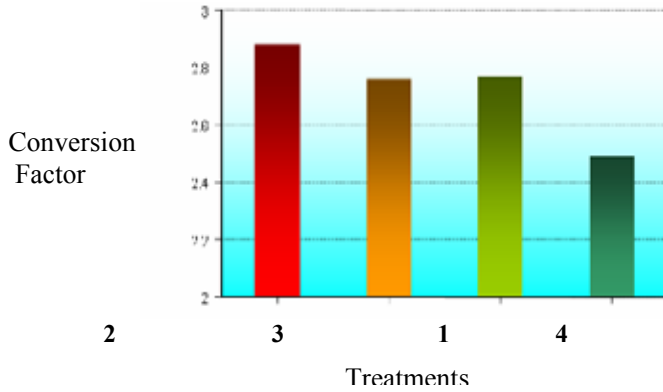
**Figure 1.** – The effect of treatments on weight gain



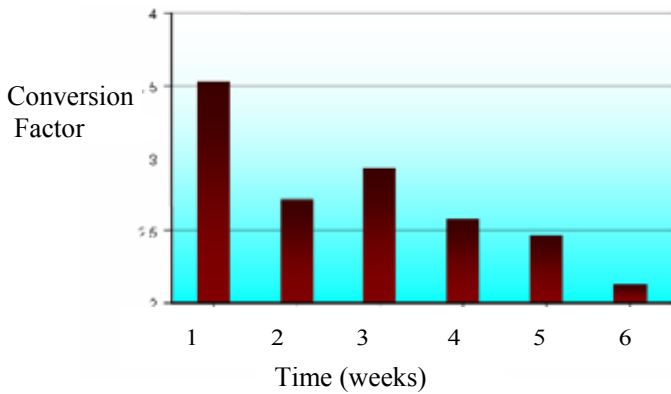
**Figure 2.** – The effect of time (weeks) on weight gain



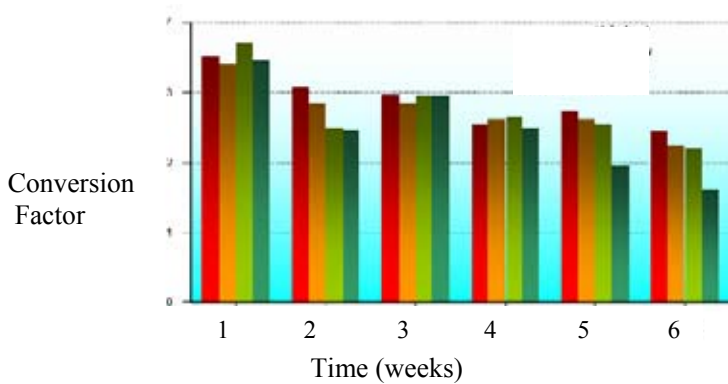
**Figure 3.** – The effect of interaction treatments and time (weeks) on weight gain



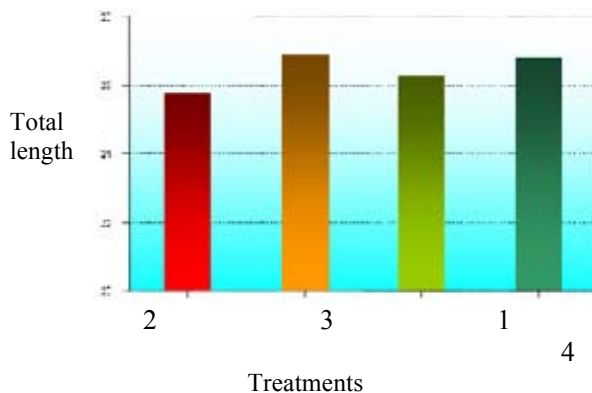
**Figure 4.** – The effect of treatments on the conversion factor



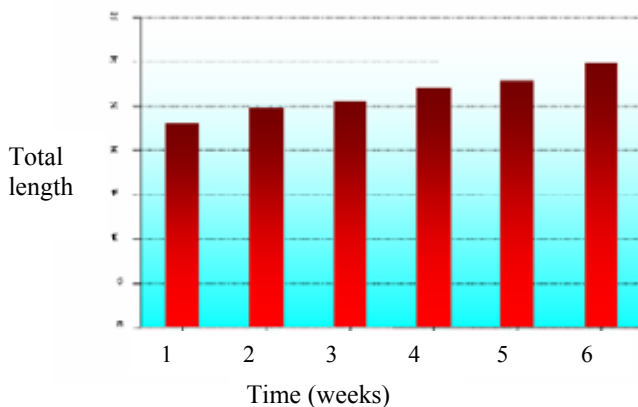
**Figure 5.**– The effect of time (weeks) on the conversion factor



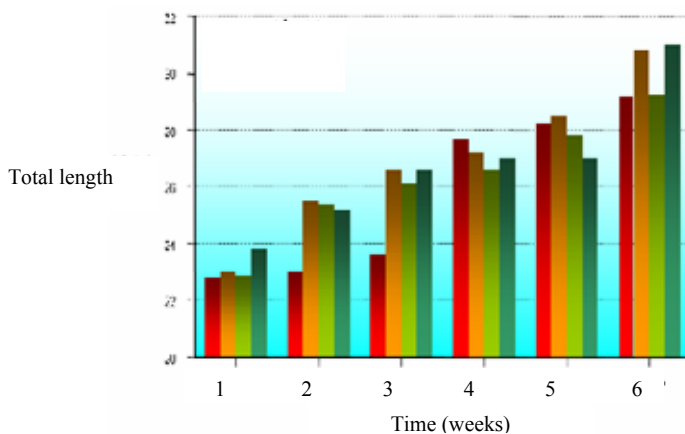
**Figure 6.** – The effect of interaction treatments and time (weeks) on FCR



**Figure 7.** – The effect of treatments on total length



**Figure 8.** – The effect of time (weeks) on total length



**Figure 9.** – The effect of interaction between treatments and time (weeks) on total length

## DISCUSSION

Cottonseed meal is high in terms of nutrition value and can be used for poultry and fish diets (Cheng et al. 2002). In this current research, we examined the usage of different amounts of cottonseed meal in rainbow trout diets. Four experimental diets were considered: common cottonseed, cottonseed with low Gossypol and cottonseed without Gossypol for feeding during a six weeks cultural period.

The diets that were consist of diverse levels of common cotton seed had significant differences ( $\alpha=0.5\%$ ) on weight increasing, total length increasing, standard length increasing and conversion factor. In comparing between different treatments, the diet which had common cotton seed had significant differences in the statically sight in weight, conversion factor, FER, total and standard length regarding the other diets and also had the worst condition.

This supports an earlier report by Robinson and Daniels (1987) that partial or complete substitution of soybean meal with cottonseed meal catfish diets resulted in similar growth, FCR, CF, survival and no Gossypol related mortality was observed. Lysine supplementation has been suggested when cottonseed meal replaced SBM completely to avoid impairing weight gain, FCR, and survival (Robinson, 1991). Fowler showed that, 34% cottonseed meal with gland was reported to induce the growth of Chinook salmon fingerlings more in comparison with 37% FM (Fowler, 1980). Coho salmon fed a diet containing 22% cottonseed meal with gland performed as well as fish fed a diet containing 37% FM.

Blom et al. (2001) reported that adult rainbow trout fed with diets in which cottonseed meal completely replaced FM had normal growth and survival percentage over the study period, but the reproductive performance of adult female trout was reduced. This was also reported by Lee et al. (2006) that female rainbow trout fertility and plasma testosterone levels were negatively affected by complete replacement of FM protein with cottonseed meal protein. In this chapter of the present study, the previously optimized levels of cottonseed meal (20%, 40% and 100%) were used as a protein source to replace soybean meal in rainbow trout diets.

A series of studies have been conducted on the utilization of cottonseed meal and toxicity of Gossypol to fish (Mbahinzireki et al. 2001, Dabrowski et al. 2000, Dabrowski et al. 2001, Blom et al. 2001). Reproductive efficiency and maternal-offspring transfer of Gossypol in rainbow trout (*Oncorhynchus mykiss*) fed diets containing cottonseed meal. However, the Gossypol level in liver was below the toxic threshold for fish fed with the experimental diets i.e., 100%, 40% and 20% cottonseed meal, indicating that Gossypol was not a barrier when feeding these diets containing Iranian cottonseed meal varieties at the levels mentioned. Moreover, this study does not support the findings of Hepher (1990) that cottonseed meal has a lower nutritional value than soybean meal.

## CONCLUSION

A few studies have been conducted with cold-water fish using cottonseed meal as a protein source and, to the best of our knowledge; this study was the first one in Iran. The results of the present study indicated that Iranian cottonseed meal varieties could be used over a period of 6 weeks (from initial weight of  $140 \pm 5$  g) to replace soybean meal without negatively affecting growth and survival of rainbow trout. In the present study, cottonseed meal varieties were used as a protein source to replace soybean meal in four



separate experiments to investigate the various inclusion level of each variety followed by comparing the optimized levels in an independent study in rainbow trout diets.

The feeding experiment in this study was conducted for a short period. Long-term studies are suggested to closely monitor Gossypol level in fillets and to ensure that Gossypol level remains in safe ranges for fish growth and survival as well as human consumption. In conclusion, cottonseed meal is a promising candidate to replace soybean meal completely in rainbow trout fish farming in Iran.

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