International Archive of Applied Sciences and Technology
Int. Arch. App. Sci. Technol; Vol 8 [2] June 2017: 62-66

[ISO9001: 2008 Certified Organization]
www.soeagra.com/iaast.html

IAAST
ONLINE ISSN 2277- 1565
PRINT ISSN 0976 - 4828

CODEN: IAASCA **ORIGINAL ARTICLE**

DOI: .10.15515/iaast.0976-4828.8.2.6266

Effect of Eugenol and Ursolic acid on Growth of Common carp, *Cyprinus carpio* under Tarai region of Uttarakhand

Adita Sharma and I. J. Singh
Dept. of Fisheries Resource Management, College of Fisheries, G. B. Pant University of Agriculture & Technology, Pantnagar

ABSTRACT
The present study was conducted for comparing the effect of Eugenol and Ursolic acid on the growth of common carp (Cyprinus carpio) under Tarai condition of Uttarakhand. The overall result of the present study revealed that role of Ursolic acid in enhancing growth, overall health, general well being and effect of Eugenol in decreasing growth in C. carpio. Higher values of CF, SCF, SGR and HSI were recorded in Ursolic acid fed fish and lower value was recorded in Eugenol fed fish. On the basis of these observations Eugenol and Ursolic acid can also be used for selective enhancement of growth depending upon the requirement.
Key words: *Eugenol, Ursolic acid, Growth, Common carp (Cyprinus carpio)*

Received 01.04.2017 Revised 10.05.2017 Accepted 29.05.2017

Citation of this article
Adita Sharma and I. J. Singh. Effect of Eugenol and Ursolic acid on Growth of Common carp, *Cyprinus carpio* under Tarai region of Uttarakhand . Int. Arch. App. Sci. Technol; Vol 8 [2] June 2017. 62-66

INTRODUCTION

Common carp is one of the highly domesticated and extensively cultured aquaculture fish species in the world. Demand for fish exceeds production and per capita consumption is steadily increasing day-by-day. To enhance fisheries production it has become a necessity to increase fisheries production by developing effective technologies for ensuring maximum output from minimum input and to get maximum profit. Strategies for increasing fish production include quality stocking material, water quality management, adequate balanced diet and incorporation of various additives. There are large number of feed additives available to improve fish growth performance some of these additives used in feed mill are chemical products especially hormones and antibiotics may cause unfavorable side effects [1]. Basil *(Ocimum basilicum* L.) is one of the most famous, annual or perennial herb belonging to the family Lamiaceae. Eugenol and Ursolic acids are the two ingredients of tulsi plant responsible for growth of fishes [4]. Eugenol is a phenolic compound and major consistuent of the essential oils extracted from different parts of Tulsi plant [2]. Ursolic acid is a triterpenoid compound which exists widely in natural plants in the form of free acid or aglycones for triterpenoid saponins [5]. Previous researches have confirmed that tulsi is cheap and easily available medicinal plant and have effect on fertility of fishes. The present study have to aim to observe the effects of the active ingredients of tulsi, Eugenol and Ursolic acid, on the growth of the fish *Cyprinus carpio.*

MATERIALS AND METHODS

Hundred specimens of common carp, average length 15.3cm and average weight 46.5g were obtained from the instructional fish farm of College of Fisheries, Pantnagar. Ten aquaria 4' x1' x1' were divided into five groups having one replicate for each group. Six specimens were put from the acclimatized fish after recording length and body weight. Diet was prepared by mixing Eugenol and Ursolic acid with the conventional diet in different concentrations as given below in the table 1. Feeding was done @ 5% body weight twice daily.

Table 1: Feeding pattern for different experimental groups of *Cyprinus carpio* during short term experiment

T_0	Conventional fish feed
T_1	Conventional fish feed + Ursolic acid@0.001 mg/kg
T_2	Conventional fish feed + Ursolic acid @ 0.00125 mg/kg
T_3	Conventional fish feed + Eugenol @ 0.06 mg/kg
T_4	Conventional fish feed + Eugenol @ 0.1 mg/kg

Short-term treatment (7days) was carried out to observe the acceptability of diet supplemented with Eugenol and Ursolic acid and its effect on survival and behavior of fish. Water quality parameters (i.e. temperature, pH, dissolved oxygen, carbon di-oxide), Survival, Feeding response and behaviour (skin coloration, movement and activity) were recorded. Long-term treatment (60days) was recorded in response to feeding of different concentrations of Eugenol and Ursolic acid for sixty days as per described feeding schedule above in table -1. The length-weight data of each specimen from all experimental groups was recorded separately at the time of first sampling. All other parameters were recorded for each group of fish separately after sixty days at the time of termination of the experiment. Paired't' test was used for comparing length and weight of common carp while Statgraphics statistical package was used for analyzing CF, SCF & SGR.

RESULTS AND DISCUSSION

Short-term treatment

Behavioural and feeding responses

The behavioural and feeding responses were shown in table 2.

Table 2. Behavioral responses of experimental fish during short-term treatment in different feeding groups

Feeding groups	**Feeding response**	**Skin coloration**	**Movement and activity**
T_0 (Conventional feed)	Optimum	Normal	Normal
T_1 (Feed + Ursolic acid@0.001 mg/kg)	Optimum	Normal	Normal
T_2 (Feed+Ursolic acid @ 0.00125 mg/kg)	More than optimum	Normal	Fast movement and activity
T_3 (Feed + Eugenol @ 0.06 mg/kg)	Optimum	Normal	Normal
T_4 (Feed + Eugenol @ 0.1 mg/kg)	Optimum	Normal	Less than normal

Feeding responsiveness and behaviour of experimental fishes were observed daily in all feeding groups. No adverse impact of Eugenol and Ursolic acid were reported on water quality parameters as well as on growth performance in all feeding groups. Maximum feeding response and activity was reported in group T_2 (Ursolic acid @ 0.00125 mg/kg) while optimum in all other feeding groups.

Water quality parameters: The temperature, Water pH, Dissolved oxygen & Free carbon dioxide (Free CO_2) have been shown in table 3.

Table 3. Temperature (°C), Water pH, Dissolved oxygen (ppm) and Free carbon dioxide (ppm) during rearing period in short term treatment with common carp

Feeding groups	Temperature				Water pH		Dissolved oxygen (ppm)		Free carbon dioxide (ppm)	
	Env. temp (°C)		Water temp (°C)							
	Period				Period		Period		Period	
	1st day	7th day	1st day	7th day	1st day	7th day	1st day	7th day	1st day	7th day
T_0 (Conventional feed)	15.0	15.5	14.0	13.5	7.0	7.0	6.1	6.0	4.1	4.0
T_1 (Feed +Ursolic acid@0.001 mg/kg)	15.0	15.5	14.0	13.5	7.0	7.0	6.1	6.2	3.9	4.0
T_2(Feed+Ursolic acid@0.00125 mg/kg	15.0	15.5	14.0	13.5	7.0	7.0	6.3	6.4	3.8	3.9
T_3 (Feed + Eugenol @ 0.06 mg/kg)	15.0	15.5	14.0	13.5	7.1	7.0	6.0	6.0	4.2	4.1
T_4 (Feed + Eugenol @ 0.1 mg/kg)	15.0	15.5	14.0	13.5	7.0	7.2	5.8	5.9	4.9	4.3

Variation in environmental and water temperature were observed during the complete experimentation period. The pH values were near neutral in all the feeding groups and did not show drastic differences among all the groups. The maximum DO value in T_2 group (6.3) during experiment. The free CO_2 concentration was slightly higher in T_3 (4.1) and T_4 (4.6) groups in comparison to rest of the groups. The maximum free CO_2 content was recorded in T_4 group (4.6). Above observations of water quality parameter indicated that there was not much difference in pH, DO or free CO_2 contents in 7 days. Maximum feeding response, activity and DO was reported in group T_2 (Ursolic acid @ 0.00125 mg/kg). Maximum CO_2 was recorded in T_4. However, pH was almost neutral in all the feeding groups.

Long-term treatment

Water quality parameters: The temperature, Water pH, Dissolved oxygen & Free carbon dioxide (Free CO_2) have been shown in table 4.

Table 4. Temperature (°C), Water pH, Dissolved oxygen (ppm) and Free carbon dioxide (ppm) during rearing period in long term treatment with common carp

Feeding groups	Temperature				Water pH		Dissolved oxygen (ppm)		Free carbon dioxide (ppm)	
	Env Temp (°C)		Water temp (°C)							
	Period				Period		Period		Period	
	Feb	Mar	Feb	Mar	Feb	Mar	Feb	Mar	Feb	Mar
T_0 (Conventional feed)	15.7	21.5	15.0	19.5	7.0	7.0	6.11	5.82	4.25	4.19
T_1 (Feed +Ursolic acid@0.001 mg/kg)	15.7	21.5	15.0	19.5	7.0	6.9	6.28	6.15	4.45	4.09
T_2 (Feed+Ursolic acid@0.00125 mg/kg)	15.7	21.5	15.0	19.5	7.0	7.0	6.68	6.50	4.50	4.0
T_3 (Feed + Eugenol @ 0.06 mg/kg)	15.7	21.5	15.0	19.5	7.0	7.1	6.01	5.90	4.45	4.15
T_4 (Feed + Eugenol @ 0.1 mg/kg)	15.7	21.5	15.0	19.5	7.2	7.0	5.53	5.32	4.80	4.25

Minimum environmental temperature in February (15.7°C) and maximum recorded in March (21.5°C). pH was almost neutral in all the experimental groups and did not show any variable trend group-wise. DO values were approximate near optimum in all feeding groups except T_3 and T_4. Free CO_2 values were approximately similar for all the experimental groups except T_4 (4.8) in which slightly higher values were reported.

All the environmental parameters are depicting that effect of ursolic acid and eugenol on growth and gonadal development. The carp culture flourishment needs a water temperature from 20-30°C but *C. carpio* is the species that can perform well at a temperature below 20°C in terms of growth and survival. Swingle [6] stated that water having a pH range of 6.5–9.0 is most suitable for carp culture. During complete experimentation period, temperature was around 20°C suitable for common carp. pH was neutral in all feeding groups and suitable for fish growth and survival. Amount of DO remained above the normal limit of 5 ppm in all feeding groups except Eugenol fed fish in which DO content was slightly lower which may be due to presence of some chemical compound present in Eugenol. Optimum concentration of free CO_2 was recorded in all feeding groups except Eugenol fed fish where slightly higher concentration was reported for which same reason may be persuaded that was responsible for less do content.

Survival

At the termination of the experiment, 100% survival was recorded in all groups, which reveals that incorporation of different ingredients in different diets had no adverse affect on the survival of fish. No abnormality was reported in behaviour and skin coloration as well as in water quality parameters when fishes were fed with diet mixed with these ingredients. Maximum feeding response was observed in the group fed with diet mixed with Ursolic acid in *C. carpio*.

Growth

(a) Body length and Body weight

The body length and body weight have been shown in table 5.

Table 5: Changes in body length (cm) and body weight (g) in long term treatment with common carp

Culture period	Treatments				
	T_0	T_1	T_2	T_3	T_4
Body length					
Initial	$16.31^{a} \pm 0.82$	$15.13^{a} \pm 0.39$	$15.26^{a} \pm 0.21$	$15.68^{a} \pm 0.25$	$14.81^{a} \pm 0.55$
Feb.	$18.37^{a} \pm 0.39$	$17.47^{ab} \pm 0.152$	$17.98^{ab} \pm 0.51$	$17.16^{b} \pm 0.44$	$17.07^{a} \pm 0.36$
March.	$18.91^{a} \pm 0.38$	$20.00^{a} \pm 0.41$	$19.17^{a} \pm 1.04$	$18.36^{a} \pm 1.46$	$18.06^{ab} \pm 0.32$
TLG	2.06	4.87	3.11	3.49	2.26
% TLG over control	--	136.40	50.48	69.41	9.70
Body weight					
Initial	$47.31^{a} \pm 0.48$	$47.20^{a} \pm 0.58$	$46.91^{a} \pm 0.41$	$46.51^{a} \pm 0.36$	$45.61^{a} \pm 1.33$
Feb.	$55.00^{a} \pm 1.98$	$60.86^{a} \pm 3.90$	$64.71^{a} \pm 4.89$	$55.9^{a} \pm 1.60$	$52.41^{a} \pm 1.80$
March.	$78.32^{a} \pm 3.68$	$80.51^{a} \pm 7.27$	$79.82^{a} \pm 7.54$	$57.33^{b} \pm 1.82$	$55.32^{ab} \pm 0.49$
NWG	31.01	33.31	32.91	6.82	19.71
% NWG over control	--	7.41	6.12	-78.00	-36.43

The TBL increased from 16.31 to 18.91 cm in T_0, 15.13 to 20.00 cm in T_1, 15.26 to 19.17 cm in T_2, 15.68 to 18.36 cm in T_3 and 14.81 to 18.06 cm in T_4. At the end of culture period, TLG was maximum (4.87 cm) in T_1 and minimum (2.06 cm) in T_0. The differences among different treatments were significant. The % TLG over control was maximum (136.40 %) in T_1 and minimum (9.70 %) in T_4. In different treatments, TBW increased from 47.31 to 78.32 g in T_0, 47.20 to 80.51 g in T_1, 46.91 to 79.82 g in T_2, 46.51 to 57.33 g in T_3 and 45.61 to 55.32 g in T_4. At the end of culture period, NWG was maximum (33.31 g) in T_1 and minimum (6.82 g) in T_3. The difference among treatments were significant. % NWG over control was 7.41 and 6.12% higher in T_1 and T_2, respectively and 78.00 and 36.43% lower in T_3 and T_4.

(b) Specific growth rate, Weight gain Condition Factor and somatic condition factor

These all parameters are shown in table 6.

Table 6: Changes in condition factor (CF), somatic condition factor (SCF), weight gain and SGR in long term treatment with common carp

Parameters	Treatments				
	T_0	T_1	T_2	T_3	T_4
Weight gain	65.54	70.57	70.15	37.66	43.21
SGR	0.36	0.38	0.38	0.11	0.26
Condition factor	0.012×10^5	0.010×10^5	0.012×10^5	0.007×10^5	0.005×10^5
Somatic condition factor	0.010×10^5	0.007×10^5	0.011×10^5	0.007×10^5	0.006×10^5

SGR was maximum (0.38) in T_2 and $T_1$and minimum (0.11) in T_3. The highest weight gain was recorded in T_1 (70.57g) followed by T_2 (70.15g) and T_0 (65.54g) and minimum in T_4 (43.21g) followed by T_3 (37.66g) group. At the end of the experiment, condition factor (K) values for fish fed with diets T_2, T_0, T_1 (0.012, 0.012 and 0.010, respectively) were found to be higher but fishes fed with T_3 and T_4 showed the lowest condition factor (0.007 and 0.005) among all the treatments. Value of SCF was greater in T_2 group (0.011) followed by T_0 (0.010) and T_1 (0.007) and lower in T_3 (0.007) and T_4 (0.006) groups.

Effect of Eugenol and Ursolic acid on body length, body weight, weight gain, SGR, CF and SCF of common carp in aquaria indicated significant gain in all parameters was recorded in T_1 (Ursolic acid@0.001 mg/kg) and T_2 (Ursolic acid @ 0.00125 mg/kg). These parameters decrease in T_3 (Eugenol @ 0.06 mg/kg) and T_4 (Eugenol @ 0.1 mg/kg). Effect of Eugenol and Ursolic acid on growth parameter showed that Ursolic acid used as growth promoter and Eugenol act as growth inhibitor.

At the termination of the experiment, hundred percent survival of fish was recorded. CF, SCF and HSI were found increasing in Ursolic acid group and decreasing in Eugenol group. CF increased as weight of fishes increased. Fishes with a high CF are relatively heavy for their length while fish with a low CF are light for their length [3]. CF and SCF were positively correlated with each other having the same pattern of variation. CF and SCF representing role of Ursolic acid in enhancing growth and energy and maintaining overall health and role of Eugenol in decreasing growth and energy. As CF is considered to be an indicator of growth, nutritional status and energy content of fish. HSI values also support the potential role of Ursolic acid in better performance of fish.

REFERENCES

1. Bello, O.S., Emikpe, B.O. & Olaifa, F.E., (2012). The Body Weight changes and Gut Morphometry of Clarias gariepinus juveniles on Feed Supplemented with Walnut (*Tetracarpidium conophorum*) Leaf and Onion (*Allium cepa*) Bulb Residues. Int. J. Morphol., 30(1), 253-257
2. Boyer, S. E., White, J. S., Stier, A. C. & Osenberg C. W. (2008). Effects of the fish anesthetic, clove oil (Eugenol), on coral health and growth, Journal of Experimental Marine Biology and Ecology, 369:53–57.
3. Chellappa, S., Huntigeord, F. A., Strangs, R. H. C. & Thomson, R. Y. (1995). Condition factor and hepatosomatic index as estimates of energy status in male three spined stickle back. J. Fish Biol., 47: 775-787.
4. Pemminati, S., Gopalakrishna, H. N. & Venkatesh, V. (2011). Anxiolytic effect of chronic administration of Ursolic acid in rats, Journal of applies pharmaceutical science, 1(03): 2011:68-71.
5. Prakash and Gupta (2004) Therapeutic uses of *Ocimum santum Linn* (Tulsi) with a note on Eugenol and its pharmacological actions: a short review. Indian j. Physiol Pharamacol 2005; 49 (2): 125-131.
6. Swingle, H.S. (1976a). Standardization of chemical analyses for waters and pond muds. FAO Fish Rep., 44 (4): 397-421.