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Effects of Feed Additive *Raafres AQ* on Feeding, Growth, Tissue Indices and Leucocytes Count in Carp Juveniles

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Effect of different levels (0, 2.5, 5, 7.5 and 10%) of feed additive *raafres AQ* on feed intake, growth, tissue indices and leucocytes count was studied in the carps *Cirrhinus mrigala* and *Labeo rohita* for a period of 28 days. The experimental diets were prepared with 35% basal diet of protein. The dietary administration of 5 and 7.5% *raafres AQ* elicited the maximum growth, tissue indices and protein efficiency ratio (PER) with low feed conversion ratio (FCR) in *C. mrigala* and *L. rohita* respectively. However, the higher doses of *raafres AQ* (7.5 – 10% in *C. mrigala* and 10% in *L. rohita*) reduced the above parameters. It suggests that lower doses of *raafres AQ* have anabolic effect stimulating feed intake, growth, tissue indices and PER and catabolic effect at higher doses causing reduction of the above parameters in chosen carps. As *C. mrigala* fed with 5% and *L. rohita* with 7.5% *raafres AQ* showed highest number of leucocytes, lymphocytes, neutrophil and monocytes; these are considered as optimum doses. *L. rohita* required a higher dose of *raafres AQ* compared to *C. mrigala.*

Keywords : *Raafres AQ*, feed intake, tissue indices, leucocyte count, carps

Incorporation of harmones, antibiotics and other additives has increased the aquaculture productivity. The growth promoting potential of anabolic steroids has been studied by many (Nirmala & Pandian, 1983; Basavaraja *et al.*, 1989; Gangadhar *et al.*, 1998). However, the fear of residual effect and consumer resistance in usage of steroid hormones prompted a search for non-hormonal growth promoters which are user friendly and of organic origin.

Traditionally, synthetic chemicals and antibiotics have been used to prevent the fish diseases and it has been achieved at least with partial success (Stoskopf, 1993). However, the emergence of antibiotic resistant microorganisms made it less successful (Anderson, 1992). Immunostimulants have been proven to be safer than chemotherapeutics and their range of efficacy is wider than vaccination (Sakai, 1998). Earlier authors have reported the growth promoting effect of hormones and synthetic feed additives on growth and immune resistance separately in various species (Nirmala & Pandian, 1983; James & Sampath, 1994; Gangadhar *et al.*, 1998; Gireesha *et al.*, 2002; Felix *et. al.*, 2004). However, the impact of feed additive *raafres AQ* on growth and leucocytes count in fishes has not been studied so far. A commercial product *Raafres AQ,* is a biodegradable and non-residual synthetic feed supplement. Manufacturers claim that *raafres AQ* reduces the load of intestinal pathogens and helps in the assimilation of feed and improves absorption of nutrients resulting better health and growth. The liquid turbogel used for binding is an excellent soft binder which prevents the wastage or leaching of feed ingredients and maintains the stability of feed. Hence, the present work has been undertaken to study the effect of feed additive *raafres AQ* on growth, tissue indices and leucocytes count in juveniles of *Cirrhinus mrigala* and *Labeo rohita.*

Materials and Methods

One hundred and fifty active juveniles of *C. mrigala* (1.41 ± 0.01g) and *L. rohita* (1.33 ± 0.09 g) were separately collected from the acclimatization tanks. *C. mrigala* juveniles were sorted out into five groups. Each group consisted of 10 individuals of more or less similar body weight and length and they

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were reared in circular cement tanks (capacity 110 l) containing 90 l water. They were maintained in triplicates. The first group served as control and were fed with pellet diet containing 35% protein. The 2nd, 3rd, 4th and 5th groups were fed with diets containing 2.5, 5, 7.5 and 10% *raafres AQ* respectively. All the experimental diets contained 35% protein. Similarly, another 5 experimental groups of *L. rohita* were maintained separately and fed with same levels of *raafres AQ* diet. The water used was clean, unchlorinated well-water and its quality was monitored biweekly (temperature : 27.0 ± 0.7^{0}C; pH: 7.8 ± 0.01; salinity: 1.18 ± 0.11$^0/_{00}$; water hardness: 307 ± 12 mg $CaCO_3$ l^{-1}; DO: 4.11 ± 0.17 ml l^{-1}). The tanks were cleaned twice a week and replenished with freshwater.

Feed additive *raafres AQ* and binder turbogel were obtained from M/s Guybro Chemical (Animal feeds, feed supplements and nutritional oral products), West Mumbai. Feed formulation was done according to Hardy (1980). The ingredients and mean composition of the diets are given in Table 1. Dried and powdered ingredients (fish meal, ground nut oil cake, vitamin and mineral mix) of diets were blended to make a homogenous mixture. Subsequently suitable levels of *raafres AQ* with binder turbogel were mixed with an aliquot of boiled water and steam cooked for 15-20 minutes. After moderate cooling, pellets (2 mm size) were

Table 1. Ingredients and mean proximate composition of experiment diets

Ingredients	g / 100 g diet
Fish meal	35
Groundnut oil cake	23
Tapioca flour	40
Cod liver oil	01
Vitamin & mineral mix	01
Proximate composition	(%)
Dry matter	92.10
Moisture	7.90
Crude protein	35.69
Crude fat	7.22
NFE	45.66
Ash	11.43

prepared with a hand operated pelletizer and dried in sunlight. After drying, diets were separately stored in refrigerator for experimental use. The protein and lipid contents of the experimental diets were determined in a spectrophotometer following Lowry *et al.* (1951) and Bragdon (1951), respectively. The moisture content was analysed by drying in an electric hot air oven at 100^{0}C. The mineral content was estimated following the method of Paine (1964). Nitrogen free extract (NFE) was calculated by subtracting the protein, lipid and mineral contents from the dry weight of the feed samples.

The experiment was conducted for a period of 28 days. The experimental fishes were fed on weighed quantity of chosen experimental diets *ad libitum* in a feeding tray daily at 0900 h and 1700 h and after one hour the unconsumed feed was collected and dried in a hot air oven at 80^{0}C for 48 h. Feed consumption or intake (mg) was estimated by subtracting the amount of unconsumed dry feed from the total dry weight of feed offered. The feeding rate was computed as:

$$\textit{Feeding rate (mg } g^{-1} \textit{ live fish day}^{-1}) = \frac{\textit{Amount of feed consumed (mg)}}{\textit{Initial wet weight of fish (g) x No. of days}}$$

The fishes were weighed at the beginning and at the end of the experiment. Growth or gain in weight was calculated as the difference between the wet weight at the beginning and at the end of the experiment. Specific growth rate (SGR) was calculated as:

$$SGR\ (\%\ day^{-1}) = \frac{\ln Wt_1 - \ln Wt_0}{t_1} \times 100$$

where, ln Wt_0 and ln Wt_1 are the weights of the fish at the beginning and at the end of the experiment and t_1 is the period between sampling in days. Feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated as:

$$\text{Feed conversion ratio} = \frac{\text{Feed consumption}}{\text{Gain in weight (g)}}$$

$$\text{Protein efficiency ratio} = \frac{\text{Wet weight gain (g)}}{\text{Protein intake (g)}}$$

Fish, feed samples and unconsumed feed were weighed in an electrical monopan balance to 1 mg accuracy. At the end of the experiment on day 28, total leucocyte count was observed in three individuals from each experimental groups. The different types of leucocytes were counted and expressed as percentage. On termination of the experiment, three individuals from each treatment were dissected out, the viscera and liver were taken, weighed and the tissue body indices were worked out as:

$$\text{Tissue indices (\%)} = \frac{\text{Weight of tissue (g)}}{\text{Weight of fish (g)}} \times 100$$

Student's *'t'* test was applied to determine the significance of mean values between the different experimental groups (Zar, 1974). ANOVA (one-way) was applied to detect significant effect of *raafres AQ* on growth parameters in *C. mrigala* and *L. rohita.*

Results and Discussion

The present study revealed that *raafres AQ* significantly (p<0.05) influenced the feeding and growth in carps (Table 2). Groups of fish treated with *raafres AQ* had higher gain in body weight than the control. The use of 5 and 7.5% *raafres AQ* resulted in higher weight gain than the rest of the treatments in *C. mrigala* and *L. rohita*. Gireesha *et al.* (2002) found that 0.5% *livol* treated *Catla catla* showed higher weight gain than control and higher doses (1 – 1.5%) of *livol.* Nacario (1983) reported that 0.1 ppm thyroxine significantly enhanced the body weight of the larvae of *Sarotherodon niloticus* than 0.5 ppm of thyroxine.

The dietary administration of 5% *raafres AQ* elicited the maximum feeding and specific growth rates (SGR) and PER while 7.5 – 10% *raafres AQ* reduced feeding, SGR and PER in *C. mrigala*. However, 7.5% *raafres AQ* produced high feeding, SGR and PER in *L. rohita*. Hence 5 and 7.5% *raafres AQ* are considered as optimum dose for *C. mrigala* and *L. rohita* respectively. It indicates that *L. rohita* required incorporation of more *raafres AQ* in diets than *C. mrigala.* Besides, an optimum dose of *raafres AQ* diet showed better performance on chosen feeding and growth parameters in *L. rohita* than in *C. mrigala*. Fish consuming 5 and 7.5% *raafres AQ* elicited the lower feed conversion ratio than other treatments. It suggests that lower doses of *raafres AQ* (5 and 7.5%) have anabolic effect stimulating feed intake and growth and catabolic effect at higher doses causing reduction in growth. Similar observation was made in young ones of the Koi carp, *C. carpio carpio* fed with frankwin and nutriwin (James *et al.,* 2007). Earlier studies revealed that livol can be used as anabolic growth promoter in common carp, *Cyprinus carpio* (Abraham, 1992), rohu, *Labeo rohita* (Maheshappa, 1994) and mrigal, *Cirrhinus mrigala* (personnel communicaion). Gireesha *et al.* (2002) found that 0.5% *livol* treated *Catla catla* showed better feed conversion efficiency and PER than control and other treatments. They also suggested that *livol* enhanced the gut digestive enzymes which in turn improved the growth rate and FCR. It is likely that, 5 and 7.5% *raafres AQ* improved the gut digestive enzymes and it resulted in more food intake, SGR and PER with less FCR in tested carps. Sambhu & Jeyaprakas (2003) reported that white prawn *Penaeus indicus* fed with 80 g kg^{-1} stafac – 20 exhibited a significantly (p<0.05) higher growth in weight, specific growth rate and RNA:DNA ratio than 0 – 60 and 100 g. kg^{-1} stafac – 20 diets. *Raafres AQ* contains â-glucan and mannon oligossacrides, which stimulates the growth of carps with less FCR.

Higher doses of *raafres AQ* (7.5 – 10% in *C. mrigala* and 10% in *L. rohita*) caused significant reduction in the rates of feeding and specific growth and conversion efficiency in chosen carps (p< 0.05). Growth reduction in carps at higher doses of *raafres AQ* may be due to the catabolic actions as reported by Lone & Matty (1983). Donaldson *et al.* (1979) found that higher doses of steroid hormones exert deleterious effects on various organs and cumulatively cancel the growth promoting effects. *Livol* at higher levels had a gradual adverse effect on growth and feed conversion efficiency as reported in rohu (Maheshappa, 1994), common carp (Abraham, 1992) and catla (Gireesha *et al.,* 2002). James *et al.* (2007) found that higher doses of *Frankmin* (6-9%) and *Nutriwin* (9%) reduced the feeding and growth parameters in Koi carp, *C. carpio carpio.*

Table 2. Effect of different levels of *raafres AQ* on chosen feeding and growth parameters in *Cirrhinus mrigala* and *Labeo rohita* reared for 28 days.

Parameters	Levels of *raafres AQ* (%)				
	0	2.5	5.0	7.5	10.0
			Cirrhinus mrigala		
Initial wet weight (g)	14.10 ± 1.22	14.25 ± 1.36	13.25 ± 1.10	13.60 ± 1.29	13.74 ± 1.33
Feed intake (g dry weight)	32.80 ± 2.86	35.11 ± 2.67	45.67 ± 4.35	43.28 ± 3.95	38.75 ± 3.12
Feeding rate (mg g^{-1} live fish day^{-1})	83.11 ± 7.69	88.01 ± 8.11	123.13 ± 10.35	113.68 ± 10.86	100.72 ± 9.75
Final wet weight (g)	18.87 ± 1.67	20.45 ± 1.77	22.66 ± 1.55	21.61 ± 2.07	21.65 ± 2.15
Weight gain (g wet weight)	4.77 ± 0.23	6.20 ± 0.45	9.41 ± 0.87	8.01 ± 0.66	7.91 ± 0.67
% weight gain (g)	34.0 ± 2.89	43.4 ± 3.64	71.0 ± 5.58	58.82 ± 3.40	58.39 ± 4.86
Specific growth rate (% day^{-1})	1.56 ± 0.15	1.82 ± 0.11	2.24 ± 0.13	2.08 ± 0.19	2.07 ± 0.14
Gross conversion efficiency (%)	14.54 ± 1.30	17.67 ± 1.39	20.61 ± 1.47	18.51 ± 1.60	20.41 ± 1.82
Feed conversion ratio	6.88 ± 0.13	5.66 ± 0.11	4.85 ± 0.29	5.40 ± 0.43	4.90 ± 0.34
Protein efficiency ratio	0.86 ± 0.01	1.14 ± 0.02	1.70 ± 0.09	1.51 ± 0.01	1.46 ± 0.05
			Labeo rohita		
Initial wet weight (g)	8.0 ± 2.61	8.05 ± 0.98	8.10 ± 0.14	8.30 ± 1.38	8.49 ± 1.37
Feed intake (g dry matter)	18.40 ± 1.69	23.40 ± 2.10	28.30 ± 2.65	34.40 ± 2.91	35.80 ± 3.25
Feeding rate (mg g^{-1} live fish day^{-1})	76.67 ± 6.80	96.81 ± 7.89	116.46 ± 9.10	138.15 ± 10.86	140.56 ± 11.45
Final wet weight (g)	12.5 ± 0.90	13.1 ± 0.30	14.2 ± 1.20	15.8 ± 1.50	17.9 ± 0.70
Weight gain (g wet weight)	4.50 ± 0.07	5.05 ± 0.19	6.10 ± 0.01	7.50 ± 0.35	9.41 ± 0.57
% weight gain (g)	50.60 ± 4.37	55.17 ± 3.56	75.31 ± 5.11	102.41 ± 5.04	110.84 ± 3.68
Specific growth rate (% day^{-1})	1.44 ± 0.05	1.57 ± 0.06	1.81 ± 0.01	2.14 ± 0.01	2.24 ± 0.30
Gross conversion efficiency (%)	22.82 ± 1.86	20.51 ± 1.10	21.56 ± 2.24	24.71 ± 2.29	26.29 ± 2.37
Feed conversion ratio	4.38 ± 0.20	4.88 ± 0.30	4.64 ± 0.20	4.05 ± 0.40	3.80 ± 0.30
Protein efficiency ratio	1.06 ± 0.02	1.37 ± 0.12	1.70 ± 0.17	2.29 ± 0.20	1.80 ± 0.08

Each value is the mean ($\overline{X} \pm SD$) of three observations

The viscerosomatic index (VSI) and hepatosomatic index (HSI) of *C. mrigala* increased with increase in *raafres AQ* levels upto a midpoint (5%) and thereafter significantly ($p<0.05$) declined (Fig. 1). However, in *L. rohita*, both VSI and HSI increased with increase in *raafres AQ* levels. Student's 't' test elicited that, there was no significant difference in VSI and HSI of *L. rohita* fed with 7.5 and 10% *raafres AQ* levels and hence 7.5% *raafres AQ* is considered as optimum level. *Raafres AQ* induced higher VSI and HSI in *L. rohita* compared to *C. mrigala* (Fig. 1). Working on white prawn *Penaeus indicus*, Sambhu & Jeyaprakas (2001) attributed that enhancement of VSI and HSI might be due to the stimulatory effect of synthetic feed additive stafac-20 on viscera and liver which supports the present study.

Supplementary feed additive *raafres AQ* significantly ($p<0.05$) influenced the leucocytes count in *C. mrigala* than in *L. rohita* (Fig. 2). Among the leucocytes, lymphocytes, neutrophil and monocytes were the predominant cell types that showed variation in response to chosen feed additive. The lymphocytes and neutrophils showed the maximum change. These two types of leucocytes are involved in the first line of defense mechanism. Administration of azadirachtin to Tilapia increased the population of macrophage and neutrophil (Logambal & Dinakaran, 2000). Sahoo (2001) found that dietary intake of levamisole (a synthetic phenylimidazolthiozole), used as an antihelminthic agent improved the non-specific immunity and disease resistance in healthy and aflatoxin induced

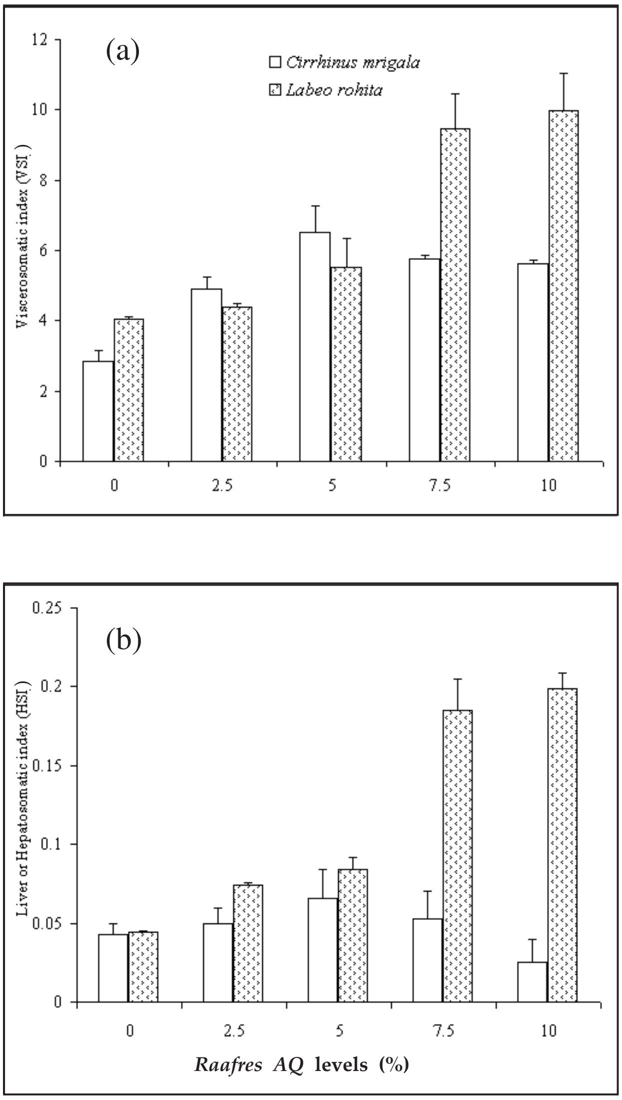

Fig. 1. Effect of different levels of *raafres AQ* on (a) viscerosomatic index (VSI) and (b) hepatosomatic index (HSI) in *Cirrhinus mrigala* and *Labeo rohita* reared for 28 days.

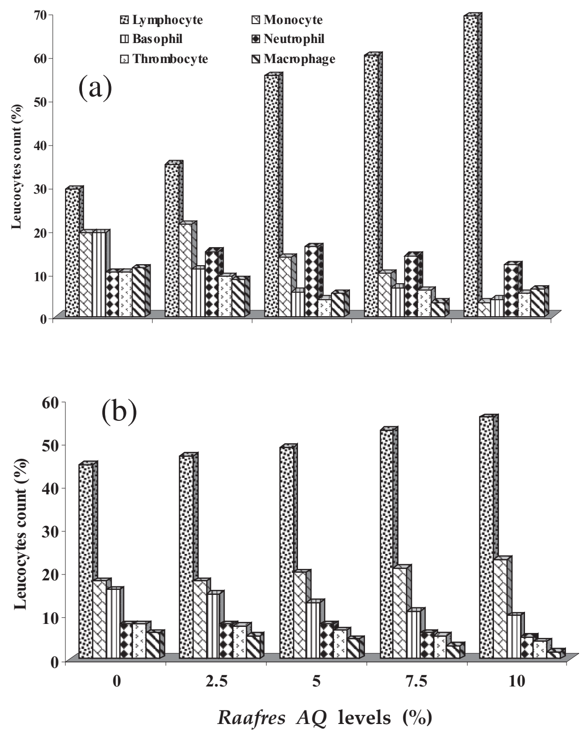

Fig. 2. Effect of different levels of *raafres AQ* on leucocytes count in (a) *Cirrhinus mrigala* and (b) *Labeo rohita*

immunocompromised rohu, *Labeo rohita*. Lymphocyte also increased in number in chosen carps and it might be due to the presence of immunostimulating compound (glucan) in *raafres AQ*. *C. mrigala* and *L. rohita* fed with 5 and 7.5% *raafres AQ* showed higher percentage of disease resistant cell types than other treatments.

The present study concludes that, *raafres AQ* at 5% in *C. mrigala* and 7.5% in *L. rohita* elicited better performance in feed intake, growth, FCR, PER, tissue indices and leucocyte counts than other treatments. Hence, 5 and 7.5% *raafres AQ* are considered as the optimum dose for *C. mrigala* and *L. rohita* respectively. Comparatively, the optimum dose of *raafres AQ* in *L. rohita* influenced percentage of weight gain, conversion efficiency and PER with less feed intake, feeding rate and FCR than the optimum dose of *raafres AQ* in *C. mrigala*. Besides, 5% *raafres AQ* incorporated diet resulted in similar impact on percentage of weight gain, conversion efficiency, FCR and PER in both carps and hence, the requirement of high dose in *L. rohita* might be nullified. Hence it is recommended to utilize *raafres AQ* in feed for better aquaculture production.

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