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Effect Of Canola Meal And Corn Gluten Based Diets On Growth, Body Composition, Blood Chemistry And Fatty Acid Profile Of Rohu (*Labeo Rohita*) At Selected Farm Sites In Punjab Province, Pakistan.

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ABSTRACT

The main hurdle in steady development of the aquaculture sector is the unavailability of balanced and cost-effective feed which accounts for almost 60% of total farming cost. To resolve this issue, current study was conducted in semi-intensive earthen ponds in district Ali Pur Chattha and Mundi Bahaudin of province Punjab (renowned aquaculture hub in Pakistan) to evaluate the effect of canola and corn gluten-based diets on the growth, body composition, hematology and fatty acid composition of *Labeo rohita*. 1000 fish stocked per acre having average weight of 198 ± 19 g at the start of trial and divided in to three groups on the stance of iso-nitrogenous and iso-caloric diets having crude protein of 30% viz. canolabased diet, corn gluten-based diet, and control diet made by 50:50 mixing of canola and corn gluten diets. Analysis of variance (ANOVA) was applied on collected data and results revealed that fish fed with canola-based diet had significantly more increase in body weight as compared to corn gluten and control diets. Significantly higher values of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as higher blood PROT and GLOB were recorded in fish of control diet. These findings indicate that *Labeo rohita* showed better growth with canola-based diet due to greater availability of protein from this source and healthy blood and fatty acid composition with control diet, respectively.

Key words: Fatty acid profile, Blood chemistry, Rohu, Canola, Corn gluten

INTRODUCTION

Fish meat represents active protein having unique composition of amino acids, a high content of polyunsaturated fatty acid viz. eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), minimum cholesterol (Calder, 2004) and fat-soluble vitamins. These micro and macro elements (Maqsood and Benjakul, 2010) are well renowned for cardio-protective (Han *et al.*, 2016), anti-thrombotic, anti-atherosclerotic and anti-arithmetic properties, respectively (Givens *et al.*, 2006). The nutritional value of fish such as fatty acid profile and taste of fish depends upon the diet (Grigorakis *et al.*, 2002) as well as region and season (Ackman *et al.*, 2002). Indian major carps including rohu (*Labeo rohita*) are major freshwater farmed species commonly found in whole Asia (Afzal Khan *et al.*, 2004) and major proportion of this fish obtained from pond culture as compared to wild (Sharma *et al.*, 2010). Rohu is mostly chosen by consumers due to is palatability, taste and lusciousness (Department, 2000) and by culturist due to its fastest growing nature, column feeding, resistance to fluctuation in physiochemical attributes of water and most importantly to temperature and salinity (Afzal Khan *et al.*, 2004).

Diet directly impacts upon the health status of fish (Ferguson *et al.*, 2010) and hematological parameters are key gauges to determine response of nutritional and anti-nutritional factors in feedstuff (Osuigwe *et al.*, 2007). However, scientist had established standard limitation of hematological characteristics of different farmed and wild fishes (Zhou *et al.*, 2009). The body condition of farmed and wild animal concerning to metabolic disorders, deficiencies and stress (Bahmani *et al.*, 2001) and ecological and biotic factors can easily be evaluated by analyzing the blood (Fernandes, 2003). Similarly, other exogenous factors such as diseases, (Yin-er *et al.*, 2005) trauma, (Cnaani *et al.*, 2004) and management (Svobodova *et al.*, 2008) persuade notable variation in blood chemistry. The major hurdle in the expansion of aquaculture sector is expensive and unbalanced feed stuff which is vital for increase in fish production (Abdel-Tawwab *et al.*, 2007). However, feed cost

in aquaculture industry includes more than 60% of total running cost (Iqbal *et al.*, 2015) depending on certain factors like source, kinds of ingredients, crude protein level and formulation practices (Glencross *et al.*, 2007) which ultimately effect the lipid profile, vitamins and mineral content of produced fish (Grigorakis *et al.*, 2002). During the present experiment the effect of two different plant source-based ingredients canola and corn gluten was evaluated on growth, body composition, hematology and fatty acid composition of *L. rohita*.

MATERIALS AND METHODS

Area of study

Trial was conducted in the earthen ponds located at two different districts, Ali Pur Chattha and Mundi Bahauddin of province Punjab, Pakistan.

Experimental design

The ponds were properly fertilized before the start of trial and each pond was stocked with 1000 *L. rohita* per acre of known body weight and body length. Fish were divided into three different treatments on the foundation of feeding with different diets made from two different kinds of plant-based ingredients viz. Canola based feed (T1) and Corn gluten-based feed (T2) having crude protein of 30% iso-caloric and iso-nitrogenous. Similarly, the control diet (mixture diet) was made by adding 50% canola-based diet and 50% from corn gluten-based diet (T0), respectively. Feeding was done at the rate of 3% of body weight, twice a day.

Water Quality Parameters

Dissolved oxygen (DO), pH, electrical conductivity, water temperature, total dissolved solids (TDS) and salinity were monitored daily twice a day. DO meter (YSI 55 Incorporated, Yellow Springs, Ohio, 4387, USA) was used to measure DO and water temperature, pH meter (LT-Lutron pH-207 Taiwan) for pH and electrical conductivity, salinity and TDS by salinity meter (Condi 330i WTW 82362 Weilheim Germany), respectively.

Growth Traits

Each fish was weighted, and its body length was measured at initiation of experiment and thereafter sampled fish on fortnightly basis to record the average increase in body weight to assess the feeds effect on the stocked fish. Other growth parameters like net gain in weight, Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) were calculated according to following formulas:

Net gain in weight (NWG) = Final body weight (g) – Initial body weight (g) Final Communication (GCP) – Final size (g) (Wet weight size (g))

Feed Conversion Ratio (FCR) = Feed given (g) / Wet weight gain (g)

Specific Growth Rate (SGR%/day) = LN (final weight) - LN (initial weight) / Number of days x 100

Proximate Analysis

The technique Near Infrared Reflectance Spectroscopy (NIRS 5000 model, Foss Tecator, Sweden) was used for proximate analysis of feed ingredients at start of trial and fish at the end. The principle of NIRS is that bonds between organic molecules absorb a specific wavelength range of light in the near infrared region. However, the feed and fish samples were dried and finely ground in pestle and mortar and then placed in sampler cups, before analysis. Values for fat, moisture, protein and ash were determined by placing cups containing samples for at least two minutes in NIRS machine (Martinez *et al.*, 2003).

Hematological and Biochemical Analysis of Blood

For hematological analysis, blood was collected at the end of experiment in vial having EDTA as an anticoagulant, while the blood for biochemical analysis was stored in the vial without anticoagulants. White blood cells (WBCs) and red blood cells (RBCs) were counted using Neubauer Haemocytometer. RBC reagent (2ml reagent/10µl blood) and WBC reagent (950µl reagents/50µl of blood) was used for counting of the respective cells in blood samples. Blood hemoglobin (Hb, g/dl), total protein (PROT, g/dl), and albumin (ALB, g/dl) of blood were estimated by Metro Lab, 1600-DR. While globulin value was determined by subtracting the values of albumin from total protein.

Preparation of Lipid Extracts and Methyl Esters

10-12 g of meat pieces of fish from three different treatments were separately minced and homogenized using a homogenizer in a solvent mixture of chloroform: methanol (2:1) kept overnight and filtered (Folch *et al.*, 1957). Anhydrous sodium sulphate is used to extract the moisture content from the lipid extract. Methyl ester of fatty acid was prepared after the extraction of lipids from the different treatments, according to (Gutierrez and Da Silva, 1993), for analyses of FAME's in the Column Gas Chromatography (GC-Model QP 2010), respectively.

Capillary-Gas Chromatography (CGC)

Analysis of FAME's was carried out on a GC, equipped with a flame ionization detector. A stainless-steel column (25×25 mm; film thickness 0.25 µm) coated with of di-ethylene glycol succinate (DEGS) was used. During operation, column temperature was kept 195°C, sample vaporizer temperature was at 225°C and detector temperature maintained at 245°C,

respectively. The injected sample size never exceeded 2-3 ml and carrier (Nitrogen) used at the rate of 40 ml/min, respectively. However, standard retention time was compared with respective chains of length for the identification of fatty acids (Ackman, 1969). Values of total fatty acid were represented in percentage of weight and peaks determined by triangulation method.

Statistical analysis

The obtained data thus subjected to statistical software SAS 9.1 and Analysis of Variance (ANOVA) was applied to compare the means and find out the relation between different parameters under study.

RESULTS

During present study physical and chemical characteristics of water such as temperature, dissolved oxygen (DO), pH, TDS, salinity, alkalinity, nitrate and electrical conductivity stayed within the optimum range as displayed in table 1.

Table 1. Physiochemical parameters of ponds water.					
Parameters	Treatment 1	Treatment 2	Treatment 0		
Salinity	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	$1.00 \pm 0.00^{\mathrm{a}}$		
pН	$8.45\pm0.35^{\rm a}$	8.31 ± 0.60^{a}	$8.08\pm0.42^{\rm a}$		
TDS	797.50 ± 245.34^{a}	710 ± 185.78^{ab}	510 ± 205.50^{b}		
EC	365 ± 162.67^{a}	308.75 ± 121.12^{a}	268.13 ± 119.94^{a}		
Temperature	$24.86\pm4.42^{\mathrm{a}}$	24.23 ± 4.16^{a}	$22.69\pm5.24^{\mathrm{a}}$		
DO	$5.58\pm0.44^{\rm a}$	6.36 ± 0.96^{a}	$6.23\pm0.73^{\text{a}}$		
Nitrate	$35\pm7.56^{\rm a}$	33.75 ± 11.88^{a}	$42.50\pm7.07^{\mathrm{a}}$		
Phosphate	$4.25\pm0.71^{\rm a}$	4.13 ± 0.64^{a}	$3.75\pm0.71^{\rm a}$		
Alkalinity	$64.50\pm17.58^{\mathrm{a}}$	53.13 ± 14.13^{ab}	47.50 ± 7.07^{b}		
Hardness	$61.88 \pm 14.62^{\mathrm{a}}$	$56.88 \pm 15.10^{\text{a}}$	$76.88\pm24.78^{\mathrm{a}}$		

Values (means \pm SD) within same row with different letters are significantly different at P<0.05.

The average stocking body weight of *L. rohita* fed with different treatments viz. T1, T2 and of T0 were 198 ± 15.33 g, 198.33 ± 19.52 g, and 197.67 ± 16.13 g, respectively. At end of trial, maximum gain in average body weight was determined in fish fed with T1 (521.67 ± 48.35 g) followed by T0 (361.66 ± 26.98 g) and T2 (350.67 ± 27.40 g) as shown in table 2. Similarly, the highest weight gain percentage 163.47 was recorded in fish fed with canola-based diet and lowest 76.81 in fish fed with corn gluten-based diet. Accordingly, minimum average value of FCR also recorded in T1 (2.28 ± 1.36) followed by T0 (2.34 ± 0.53) and T2 (2.62 ± 0.81), respectively.

Table 2. Growth parameters of Labeo rohita fed with canola (T1), corn gluten (T2) based and mixed (T0) diets.

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Treatments	Weight Gain (g)	Weight Gain %	FCR	SGR%
T1	521.67 ± 48.35^{a}	163.47	2.28 ± 1.36^{a}	$1.67\pm0.65^{\rm a}$
T2	350.67 ± 27.40^{b}	76.81	2.62 ± 0.81^{a}	1.13 ± 0.32^{a}
T0	361.66 ± 26.98^{b}	82.96	2.34 ± 0.53^{a}	$1.28\pm0.35^{\rm a}$

Means \pm SD with different letters in a column are statistically significant at P<0.05.

While the average values of SGR% different treatments such as T1 (1.67 ± 0.65), T2 (1.13 ± 0.32), and T0 (1.28 ± 0.35) are shown in table 2. To evaluate the impact of different diets on body composition of *L. rohita*, proximate analysis of fish was done at the end of trial as shown in table 3. The lowest average value for crude protein percentage (CP%) was recorded 66.25 ± 3.10 in treatment T2 while highest 70.40 ± 2.51 found in fishes fed with treatment T1, and 69.37 ± 2.51 found in T0, respectively. However, non-significant difference was observed for average value of fat in T1 (3.36 ± 0.44) and T0 (3.25 ± 0.47) while lowest values noted in T2 (2.94 ± 0.48). The average values of ash content for the fishes fed with treatments T1, T2 and T0 during study was recorded 18.36 ± 1.92 , 19.40 ± 1.69 , and 19.83 ± 2.52 , respectively. The maximum average value of dry matter % found in T1 (25.47 ± 2) while lowest noted in T2 (20.57 ± 2.79) as shown in table 3, respectively.

Table 3. Body composition of <i>Labeo rohita</i> at the end of trial fee	l with different f	eeds.
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Parameters	Treatment 1	Treatment 2	Treatment 0
Crude protein %	$70.40\pm2.51^{\mathrm{a}}$	66.25 ± 3.10^{b}	69.37 ± 2.51^{a}
Fat %	3.36 ± 0.44^{a}	2.94 ± 0.48^{b}	3.25 ± 0.47^{ab}
Dry matter %	$25.47\pm2^{\rm a}$	20.57 ± 2.79^{b}	21.07 ± 3.13^{b}
Ash %	$18.36 \pm 1.92^{\text{a}}$	19.40 ± 1.69^{a}	19.83 ± 2.52^{a}

Means \pm SD with different letters in same row are statistically significant at P<0.05.

The biochemical parameters of blood such as WBC (103/µl), RBC (106/µl), Hb (g/dl), ALB (g/dl), GLOB (g/dl) and PROT (g/dl) of fish fed different treatment viz., T1, T2, and T0 are given in table 4. For the average values of WBC's,

non-significant difference was observed while slight significant difference found for the average values of RBC's such as 1.26 ± 0.16 was recorded T1, 1.45 ± 0.20 in T2 and in T0 1.66 ± 0.15 was determined, respectively.

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Parameters	Treatment 1	Treatment 2	Treatment 0	
WBC (10 ³ /µl)	$14.77\pm0.78^{\rm a}$	$13.93\pm0.96^{\mathrm{a}}$	$15.02\pm1.32^{\rm a}$	
RBC (10 ⁶ /µl)	1.26 ± 0.16^{b}	1.45 ± 0.20^{ab}	$1.66\pm0.15^{\rm a}$	
Hb (g/dl)	6.34 ± 0.10^{b}	6.48 ± 0.25^{ab}	$6.63\pm0.12^{\rm a}$	
PROT(g/dl)	10.34 ± 0.17^{b}	10.29 ± 0.13^{b}	$10.66\pm0.30^{\mathrm{a}}$	
ALB(g/dl)	$5.48\pm0.11^{\text{b}}$	5.69 ± 0.20^{b}	$6.02\pm0.22^{\rm a}$	
GLOB(g/dl)	4.86 ± 0.13^{a}	$4.60\pm0.18^{\rm a}$	4.64 ± 0.32^{a}	

Table 4. Biochemical evaluation of blood of *labeo rohita* from different treatments at the end of trial.

Means \pm SD within same row with different letters are significantly different at P< 0.05.

Although, the highest maximum average value of hemoglobin 6.63 ± 0.12 was recorded in T0 and minimum 6.34 ± 0.10 was determined in T1. Similarly, highest value of total protein 10.66 ± 0.30 in blood determined in T0 while non-significant difference observed between T1 (10.34 ± 0.17) and T2 (10.29 ± 0.13), respectively. The average values of albumen (ALB) in treatments T1, T2 and T0 was recorded 5.48 ± 0.11 , 5.69 ± 0.20 and 6.02 ± 0.22 while 4.86 ± 0.13 , 4.60 ± 0.18 and 4.64 ± 0.32 were average values of globulin (GLOB) determined for T1, T2 and T0 as shown in table 4, respectively.

The fatty acid profile of fish meat of different treatments was determined by collecting ten sample from each groups as shown in table 5. Significant difference was observed for the average values of lauric acid (C-12:0) among treatments T1 (0.146 ± 0.030), T2 (0.069 ± 0.038), and T0 0.198 ± 0.049 , while non-significant difference determined for the average values of tridecylic acid (C-13:0) among the different treatments, respectively. Similarly, no significant difference was determined for myristic acid (C-14:0) between the fishes of treatments T1 (6.447 ± 0.786) and T0 (7.040 ± 0.883), but lowest level found in T2 (3.202 ± 1.575). Accordingly, the lowest average value (0.699 ± 0.261) of pentadecylic acid (C-15:0) found in T2 as compared to T1 and T0 (1.460 ± 0.212 and 1.915 ± 1.217).

Table 5. Determination of fatty acid profile of muscle of rohu at the end of trial fed with different diets.

Fatty Acids	Treatment 1	Treatment 2	Treatment 0
C-12:0	0.146 ± 0.030^{b}	$0.069 \pm 0.038^{\circ}$	0.198 ± 0.049^{a}
C-13:0	$0.146\pm0.028^{\mathrm{a}}$	0.066 ± 0.044^{a}	$0.350\pm0.618^{\mathrm{a}}$
C-14:0	6.447 ± 0.786^{a}	3.202 ± 1.575^{b}	$7.040\pm0.883^{\mathrm{a}}$
C-15:0	$1.460 \pm 0.212^{\rm a}$	0.699 ± 0.261^{b}	$1.915 \pm 1.217^{\mathrm{a}}$
C-16:0	28.720 ± 1.292^{a}	21.242 ± 7.760^{b}	27.910 ± 2.246^{a}
C-17:0	$3.080\pm0.394^{\mathrm{a}}$	$2.252\pm0.831^{\text{b}}$	$2.870\pm0.368^{\mathrm{a}}$
C-18:0	$11.640 \pm 1.654^{\mathrm{a}}$	6.652 ± 3.348^{b}	10.512 ± 2.407^{a}
C-16:1-n9	4.790 ± 0.522^{a}	2.672 ± 1.142^{b}	$4.910\pm2.483^{\mathrm{a}}$
C-18:1-n9	$6.950 \pm 0.973^{\rm a}$	4.222 ± 1.945^{b}	$8.020\pm2.867^{\mathrm{a}}$
C-18:1-n7	2.340 ± 0.366^{b}	1.432 ± 0.538^{c}	$2.810\pm0.739^{\text{a}}$
C-20:1-n9	0.580 ± 0.151^{b}	$0.217 \pm 0.079^{\circ}$	$0.841 \pm 0.311^{\rm a}$
C-22:1-n9	0.289 ± 0.066^a	0.095 ± 0.097^{b}	0.336 ± 0.202^{a}
C-18:2-n6	10.360 ± 1.271^{a}	6.012 ± 2.839^{b}	10.880 ± 2.009^{a}
C-18:3-n6	0.566 ± 0.097^{a}	0.216 ± 0.196^{b}	0.464 ± 0.170^{a}
C-20:2- n6	0.604 ± 0.107^{a}	0.317 ± 0.125^{b}	0.574 ± 0.044^{a}
C-20:3- n6	$0.523\pm0.108^{\mathrm{a}}$	0.268 ± 0.096^{b}	$0.525\pm0.212^{\text{a}}$
C-20:4- n6	$6.860 \pm 1.568^{\rm a}$	3.822 ± 1.529^{b}	$5.980\pm0.919^{\mathrm{a}}$
C-20:4-n3	$0.456 \pm 0.093^{\mathrm{a}}$	0.277 ± 0.083^{b}	$0.446\pm0.083^{\text{a}}$
C-20:5-n3(EPA)	1.144 ± 0.267^{a}	0.717 ± 0.237^{b}	$1.261\pm0.240^{\mathrm{a}}$
C-22:4-n6	$0.340 \pm 0.039^{\mathrm{a}}$	0.227 ± 0.063^{b}	0.379 ± 0.091^{a}
C-22:5-n3	$2.107\pm0.476^{\mathrm{a}}$	1.312 ± 0.564^{b}	$2.247\pm0.399^{\mathrm{a}}$
C-22:6-n3(DHA)	8.760 ± 1.130^{b}	$4.562 \pm 2.003^{\circ}$	10.910 ± 1.711^{a}

Means \pm SD with different letters in same row are statistically significant at P<0.05.

For palmatic acid (C-16:0), significant lower values are determined in T2 (21.242 ± 7.760) as compared to T1 (28.720 ± 1.292) and T0 (27.910 ± 2.246), respectively. Similarly, the average values of margaric acid (C-17:0) of the fish fed with T1 (3.080 ± 0.394) was recorded higher as compared to T2 (2.252 ± 0.831) and T0 (2.870 ± 0.368), respectively. The highest average value of stearic acid (C-18:0) was calculated 11.640 ± 1.654 for T1 and minimum was seen (6.652 ± 3.348) in fished fed with treatment T2, respectively. There was no major difference was observed among T1 and T0 for the values of palmitoleic acid (C-16:1-n9). The minimum figures (2.672 ± 1.142) of palmitoleic acid was recorded for fish fed with T2 based diets, respectively. There was significant difference was seen among the values of different treatments regarding to oleic acid (C-18:1-n9) and vaccenic acid (C-18:1-n7). The average value of gondoic acid (C-20:1-n9) for T1,

T2 and T0 was recorded 0.580 ± 0.151 , 0.217 ± 0.079 and 0.841 ± 0.311 , respectively. However, the maximum average value of erucic acid (C-22:1-n9) was recorded 0.336 ± 0.202 in T0 and minimum 0.095 ± 0.097 was measured in fish fed with T2 diets. The minimum value of linoleic acid (C-18:2-n6) was recorded 6.012 ± 2.839 in the fish fed with T2 diets and slight difference was observed between the values of T1 (10.360 ± 1.271) and T0 (10.880 ± 2.009). The average value of γ -linolenic acid for T1 was recorded 0.566 ± 0.097 , T2 was calculated 0.216 ± 0.196 and for T0 was found 0.464 ± 0.170 , respectively.

The highest average value 0.604 ± 0.107 of eicosadienoic acid (C-20:2-n-6) was found in T1 and lowest value 0.317 ± 0.125 observed in T2 while 0.574 ± 0.044 found in T0. However, the average value of dihomo-gamma-linolenic acid {DGLA (C-20:3-n-6)} viz., 0.523 ± 0.108 , 0.268 ± 0.096 and 0.525 ± 0.212 determined in treatments T1, T2 and T0, respectively. The maximum average value of arachidonic acid {AA (C-20:4-n-6)} was recorded maximum in T1 (6.860 ± 1.568) followed by T0 (5.980 ± 0.919) and T2 (3.822 ± 1.529), respectively. There was non-significant difference was observed for the value of eicosapentaenoic acid {EPA, Timnodonic acid (20:5-n3)} among different treatments while significant difference noted for the average value of Eicosatetraenoic acid {ETA (20:4-n-3)} and lowest observed in T2 (0.277 ± 0.083) as shown in table 5. There was significant difference was also observed between the values of adrenic acid (22:4-n6) and docosapentaenoic acid{DPA, Clupanodonic acid (22:5-n3) among different treatments. The average value of adrenic acid was recorded 0.340 ± 0.039 , 0.227 ± 0.063 and 0.379 ± 0.091 for treatments T1, T2 and T0 and the average values of docosapentaenoic acid {DHA, Cervonic acid (22:6-n3)} was recorded 10.910 ± 1.711 for T0 and minimum was for T2 4.562 ± 2.003 , respectively. However, the values of DHA for treatment T1 was recorded 8.760 ± 1.130 , during the course of present experiment as shown in table 5.

DISCUSSION

During present study physical and chemical characteristics of water such as temperature, dissolved oxygen (DO), pH, TDS, salinity, alkalinity, nitrate and electrical conductivity stayed within the optimum range and non-significant differences were observed among all treatments and in accordance with previous findings (Abbas *et al.*, 2008; Iqbal *et al.*, 2014). During present trial, significant difference at P<0.05 was observed for the average values of body weight gain among different treatments viz. T1, T2, and T0, respectively. Results are in agreement with (Iqbal *et al.*, 2015), who found that *L. rohita* showed more gain weight treated with plant-based feed ingredients as compared to fish meal, while contradictory results were observed when canola meal replaced with fish meal in major carps (Abbas *et al.*, 2008). However, non-significant difference in the growth and body composition of *Cyprinus carpio* treated with different agro based waste material as compared to fish meal (Hasan *et al.*, 1997), while increase in body weight of fish recorded with increasing the level of crude protein in diet of fish (Abid and Ahmed, 2009). It has been confirmed by present study that crude protein is not only factor which affect the growth properties of fish but other factors also affect such as rearing conditions and so on (Miroslav *et al.*, 2011). However, non-significant results were analyzed for the values of FCR and SGR. Although the FCR values were very high at start of trial but can be justified with previous results which state that decrease in FCR with increase in size (Iqbal *et al.*, 2015).

The results of proximate analysis of fish during current study were in accordance with the previous findings (Umer and Ali, 2009, Iqbal et al., 2015), who observed significant variations in fat content of meat when matched with different sources-based diets such as animal and plant in *L. rohita* while non-significant results for protein content. There were non-significant changes were estimated for the concentration of WBCs (103/µl) and slightly significant changes were noted for the concentration of RBCs (106/µl) and blood PROT among all treatments. The concentration of RBCs was in direct proportionate to the blood PROT value of different treatments, while increase in RBC's observed with increasing protein level of diet (Nasir and Al-Sraji, 2013). However, decrease in WBCs and RBCs are also noted with increase in protein of diet (Iqbal *et al.*, 2014; Yue and Zhou, 2008). The higher blood PROT concentration recorded in T1 fed with canola meal based diet might represent more availability of protein from this source to fish. Significant differences were recorded for the values of blood PROT in larvae fed with artificial diet (Yousefian *et al.*, 2013).

Based on lipid contents in the muscle, fish are divided into four major classes viz., very low fat (<2% fat), low fat (2–4% fat), medium fat (4–8% fat), and high fat (>8% fat) (Ackman, 1994), while carps recognized as low fat fish (Sen, 2005). Fishes derived fatty acids from two main sources; first from diet and secondly by biosynthesis (Kamler *et al.*, 2001), while ratio of favorable polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) found in carps fed with complete diet as compared to wheat and maize (Miroslav *et al.*, 2011). That's why, feed directly affect the composition of fish (Steffens and Wirth, 2007). Higher concentration of fatty acid with no double bond viz. C-14:0, C-18:0 and especially C-16:0 was found in present study while similar results were found by previous studies (Sharma et al., 2010; Ackman, 1994; Gutierrez and Da Silva, 1993). (Swapna *et al.*, 2010) observed EPA value of 1.6% in *L. rohita* and 2.1% in *Catla catla* while low values also recorded during present study, accordingly. The values of DHA are in line with the results of (Ackman, 1994) and (Swapna *et al.*, 2010), who recorded up to 7.6% in all freshwater species except *C. catla*.

CONCLUSIONS

Due to higher availability of protein and micronutrients from canola based diet led to highest gain in body weight. While stagnant growth in fish fed with corn gluten-based diet is due to presence of anti-nutritional factors in it. While stable fatty

acid profile and healthy blood profile determined in fish fed with mixed feed influence the importance of balance diet. It had been concluded that protein is not the only factor which effect the growth of fish but others such as lipid content, feed taste and acceptability to fish, quality of feed, water quality and management of ponds as well. There is dire needs to evaluate the impact of other factors for the efficiency of *L. rohita* culture sector.

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