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Changes in biochemical composition in flesh of common carp, *Cyprinus carpio* (L.) fingerlings, FED with diets replacing soybean with fish silage protein at different levels

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Abstract
The present study was conducted to evaluate the changes in biochemical composition of flesh of the common carp fingerlings fed with diets replacing protein of plant origin i.e. soybean with animal protein in the form of fish silage at different levels. Five experimental diets (D_1 – D_5) were prepared to replace soybean meal with fish silage @ 15% (D_2), 30% (D_3), 45% (D_4) and 60% (D_5) against control (D_1) diet. No significant difference in water quality recorded w.r.t incorporation of fish silage in the diets and all basic water quality parameters were within suitable range. In present study, protein assimilation recorded maximum at 34.98% (D_4). Among biochemical parameters of fish flesh, moisture content ranged between 78.20 – 81.43%, crude protein 13.90 – 16.50%, fat1.60 – 2.50%, NFE 1.10 – 1.50%, crude fibre 0.30 – 0.36% and ash 1.06 – 1.60%. In fish flesh, accumulation of protein content was significantly improved when fishes were fed with diets replacing soybean meal with fish silage protein @ 45% (T_4).

Keywords: Common carp, *Cyprinus carpio*, soybean protein, fish silage protein, biochemical composition

Introduction
Feed is one of the major recurring inputs in farming systems of livestock; hence fish culture is not an exception to it. Balanced diet is an essential pre-requisite for good production but due to its cost and quantity expenditure on feed some time shares more than 60% of the total production cost [1]. In fish farming, where rearing of fish seed is done at higher stocking rate, hence requires more quantity of food, however nutrient present in pond water are not getting optimum time to be converted in to natural fish food organisms as; the practice of flushing out of excessive metabolites through water exchange is being done to maintain water quality. Such water quality management practices leads to scarcity of natural fish food organism; hence it becomes mandatory to feed the fish with supplementary diets. In fish culture system, fetching higher production is possible only through providing nutritionally balanced diet, given at the right time; in optimum quantity. Balanced diet containing all desired nutrients at optimum levels; helps in attaining fast growth, better health and optimum reproductive performance. Conventionally, oil cake and rice bran is the most common, locally available, cheapest feed ingredient being used in carp feed. Soybean meal is one of the most commonly used feed ingredient of plant origin protein source, due to its easy availability and richness in essential amino acids [2], while the fish meal is the preferred source of dietary animal protein for many fish species as it contains all essential amino acids with high digestibility [15].
Common carp (*Cyprinus carpio L.*) is an important fresh water carp, cultured across the world because of its hardy nature, wide acceptability of feed ingredient and easy breeding. At present common carp is being reared in almost all parts of the country as a major commercial fish species. Significance of the nutrient balance in feed is more as its requirement changes with transition of life stage from one stage to another stage and accordingly preference of food and nutritional requirement also changes. Early stages of the life cycle of any organism are very crucial for its survival and development, similarly common carp requires an optimum amount of dietary protein. Processed waste from aquatic as well as terrestrial animals is considered as an excellent source of protein as it is enriched with lysine, sulfur amino acids (methionine and cysteine), histidine, arginine, phosphorous [5]. Fish meal is the major source of dietary protein, but its production involves high investment in its processing plant setup and expenditure on energy. Fish silage can be an alternative source of animal protein.

The current study was conducted to evaluate diets replacing soybean meal with fish silage protein @ 15, 30, 45 and 60% as source of protein and to observe the changes in biochemical composition of fish flesh in response to optimum combination for replacement in fingerlings of common carp, *Cyprinus carpio*.

2. Material and Methods

The experiment was conducted in the FRP tanks (1.5×1.0×0.75m) for 90 days at the College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab). Five experimental diets (D_1– D_5) were prepared by adding rice bran @38%, mustered meal @ 30%, carboxy methyl cellulose @ 2%, mineral mixture @ 2%, vitamin mixture @ 2% along with soybean meal 26% in D_1 (control), soybean meal @ 22.1% and fish silage @ 3.9% in D_2, soybean meal @ 18.2% and fish silage @ 7.8% in D_3, soybean meal @ 14.3% and fish silage @ 11.7% in D_4 and soybean meal @ 10.4% and fish silage @ 15.6% in D_5. Experiment was conducted in triplicate for all experimental diets. Each tank was stocked with pre-acclimatized 13 fingerlings of common carp (*C. carpio*) per pool. Average weight of the fingerlings ranged between 5.41 – 5.49 g, while length ranged 6.61 – 6.74 cm. Fish silage used in the present study was prepared as per the modified method described using 3% formic acid for proteolytic activity and preservation [5]. Fish were fed with respective diets @5% body weight split into two equal doses, twice a day. Proximate composition of feed was evaluated as per standard method [6], while proximate composition of flesh was observed for total protein [7], total lipid [8], moisture and ash [6]. Water quality was tested as per standard methods [9].

Data recorded for water quality, biochemical composition of flesh, two-tailed bivariate Pearson's correlation coefficient were statistically analyzed for average values, standard deviation and correlation coefficient using one way ANOVA by SPSS 16.00 software.

3. Results and Discussion

Water quality is an important criterion for the survival, health and growth of aquatic organism. Though, the supplementary feed improves fish growth but its nutrient composition, digestibility of feed ingredients along with physical properties, unutilized feed and excretory matter causes significant effect on water quality [10, 11]. During the present study, water quality with respect to temperature, pH, dissolved oxygen, alkalinity, hardness, ammonia, nitrite and orthophosphate in different treatments were recorded within the desirable range for fish culture; as suggested for warm water fish species [12, 13]. Details of the water quality recorded during experimental period are given in Table 1. No significant difference in water quality recorded w.r.t. addition of fish silage to replace soybean protein at different level.

All five experimental diets (D_1-D_5) were analyzed for its proximate composition on dry matter basis and recorded highest crude protein diet in D_5 (37.04%), whereas lowest protein content recorded in the D_1 (29.32%). Fat content in diets ranged between 3.46 (D_1) – 5.08% (D_5), as minimum and maximum. Details of the proximate composition of experimental diets are given in Figure 1. Diets replacing soybean meal with fish silage as source of protein were prepared and fed to fishes to observe the changes in biochemical composition of fish flesh in response to optimum combination for replacement.

Table 1: Water quality parameters (Average mean values during experimental period.

Parameters \ Treatments	D_1	D_2	D_3	D_4	D_5
Water Temperature (ºC)	26.10^{a}±1.04	26.16^{a}±1.087	26.10^{a}±1.067	26.12^{a}±1.111	26.14^{a}±1.175
pH	8.73^{a}±0.036	8.68^{a}±0.106	8.76^{a}±0.057	8.83^{a}±0.050	8.75^{a}±0.055
Dissolved Oxygen (mg l^{-1})	8.69^{a}±0.116	8.64^{a}±0.106	8.69^{a}±0.124	8.67^{a}±0.091	8.67^{a}±0.151
Total alkalinity ($CaCO_3$mg l^{-1})	280.94^{a}±2.93	282.75^{a}±3.37	280.92^{a}±4.01	280.08^{a}±2.90	281^{a}.22±3.17
total hardness ($CaCO_3$mg l^{-1})	291.08^{ab}±2.17	293.20^{a}±3.33	295.58^{a}±2.10	297.11^{a}±3.19	296.40^{a}±3.17
Water ammonia (mg l^{-1})	0.0056^{a}±0.0003	0.0054^{a}±0.0003	0.0057^{a}±0.0003	0.0053^{a}±0.0003	0.0055^{a}±0.0003
Nitrite (mg l^{-1})	0.045^{a}±0.002	0.045^{a}±0.004	0.047^{a}±0.004	0.046^{a}±0.003	0.044^{a}±0.002
Orthophosphate (mg l^{-1})	0.051^{ab}±0.003	0.052^{ab}±0.001	0.059^{a}±0.001	0.058^{a}±0.001	0.059^{a}±0.001

*Values with different alphabetical superscripts differ significantly within a row ($P \leq 0.05$)

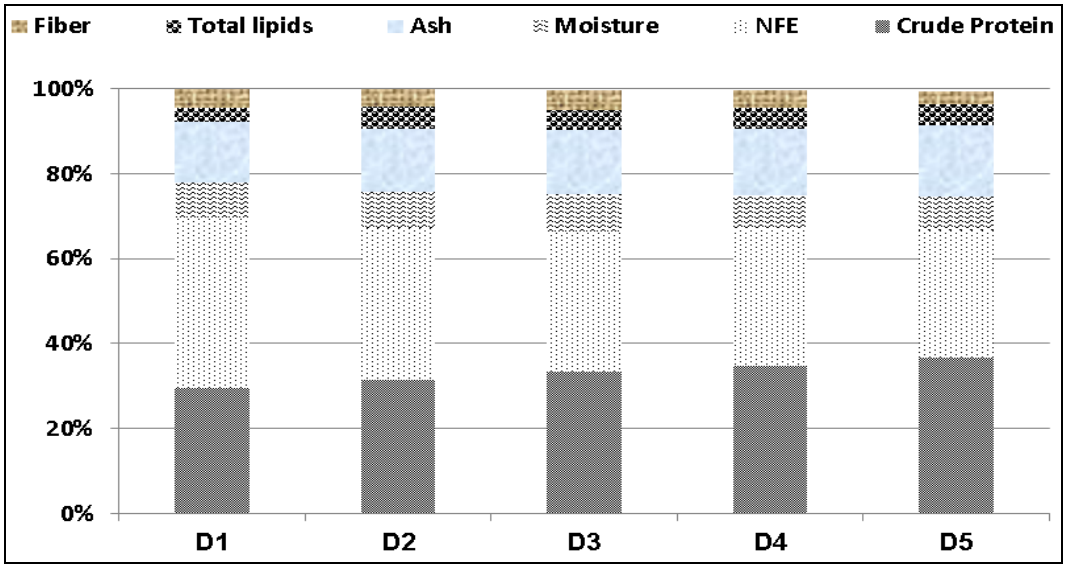

Fig 1: Proximate composition (%) of formulated diets (on DM basis).

It is suggested that 25 – 36% crude protein as optimum level in diets for warm water fishes, however protein requirement varies with species, size, sex, biological condition, health status and environmental factors [14]. In common carp juveniles, the highest growth reported, in group of fish when fed with 30% crude protein diet, as compared to 25 and 35% protein level diets [15]. It is also suggested the protein requirement in carp fish is in-between 25-35% [3]. In the present study, protein content in the experimental diets prepared to feed the fish was almost in the range of protein requirement suggested by different workers.

Flesh composition of fish was analyzed for moisture, protein, lipid, ash, Nitrogen free extract (NFE) and fiber, at the time of start and termination of experiment. Significantly ($P \leq 0.05$) high moisture content was recorded in the flesh of fish fed with experimental diet D_2 and D_3 other than at the time of stocking. Protein (%) content in the fish flesh in all treatments (D_1 to D_5) ranged in-between 13.90 – 16.50. Significantly ($P \leq 0.05$) high protein content recorded in the flesh of fish fed with diets D_4 and D_5, whereas significantly ($P \leq 0.05$) high lipid content (%) recorded in flesh of fish fed with diet D_5. Ash content (%) in fish flesh in all treatments (D_1 to D_5) varied significantly ($P \leq 0.05$) and ranged in-between 1.06 to 1.60, while carbohydrate as NFE (%) ranged in-between 1.10 to 1.50 in the flesh of fish fed with diet D_1–D_5. Details of the biochemical composition of fish flesh w.r.t. different experimental diets are given in Table 2.

Table 2: Biochemical composition (%) of common carp flesh (Mean±SE).

Parameter (%)	Treatments					
	Initial	D_1	D_2	D_3	D_4	D_5
Moisture	83.43^{a}±0.68	81.32^{a}±0.28	81.43^{a}±0.12	80.44^{b}±0.05	78.20^{b}±0.05	78.40^{b}±0.05
Proteins	12.66^{c}±0.08	13.90^{b}±0.05	14.10^{b}±0.05	14.20^{b}±0.05	16.50^{a}±0.05	16.01^{a}±0.05
Lipids	1.33^{b}±0.23	1.60^{b}±0.05	1.83^{b}±0.06	1.90^{b}±0.05	2.10^{a}±0.05	2.50^{a}±0.05
Ash	1.06^{c}±0.03	1.06^{c}±0.08	1.20^{b}±0.05	1.20^{b}±0.05	1.60^{a}±0.05	1.23^{b}±0.03
Crude fiber	0.38^{a}±0.03	0.30^{b}±0.05	0.33^{b}±0.05	0.32^{b}±0.05	0.36^{a}±0.05	0.30^{b}±0.05
NFE	1.16^{b}±0.09	1.20^{b}±0.05	1.10^{b}±0.05	1.50^{a}±0.05	1.20^{b}±0.05	1.40^{a}±0.05

*Values with different alphabetical superscripts differ significantly within a row ($P \leq 0.05$)

Biochemical studies of fish tissue are of considerable interest for their specificity in relation to the food values of the fish and for the evaluation of their physiological needs at different phase of life. Food composition, life stage, sex, environment and genetic trait are well known factors responsible to influence chemical composition of fish flesh [16]. The percentage composition of the four major constituents of fish viz. water, protein, lipid and ash (minerals) is referred to as proximate composition. These four components account for about 96-98% of total tissue constituents in most cases. The principle constituents are water (66 – 84%), protein (15 – 24%), lipids (0.1 –22%), minerals (0.8 – 2%) and carbohydrate in very minute quantity (0.3%) at maximum value in fishes [17]. The proximate composition of Indian fishes ranges between 65 – 90% water, 10 – 22% protein, 01 – 20% lipid and 0.5–05% minerals [18]. It is also reported that the moisture content 77.2 – 82.4, protein content 13.60 – 16.7, lipid content 1.75 – 2.73 and ash content 1.40 – 2.61, when fed with diet containing 35 – 39% protein in the fingerlings of common carp (*C. carpio*), and the values recorded in the present investigation are quite near to the values reported by different scientists [19]. The result indicates that the assimilation of protein in flesh increases until it reaches to the optimum level, beyond that both its digestion as well as assimilation declines; hence goes as waste. In present study, assimilation of protein recorded maximum at 34.98% (D_4). It is also suggested that in common carp temperature and metabolizable energy affects the protein requirement for promoting growth [10]. It is suggested that excess protein in diet w.r.t. desirable levels does not absorb and accumulate efficiently; hence goes as waste and also causes stress to fish, similarly excess energy is known to induce lipogenesis thus necessitating a balance between protein and energy in diet formulation [3].

Biochemical compositions of the fish flesh on the pooled data basis were compared for two-tailed bivariate Pearson's correlation coefficient (Table 3). The results indicate that the moisture content showed negative correlation with protein, lipid, carbohydrate and ash, whereas positive correlation with fiber. Protein has positive correlation with lipid and carbohydrate whereas protein, lipid and carbohydrate showing negative correlation with fiber. The positive correlation among protein, lipid and NFE content may be due to growth stage, more over the fishes were kept under controlled and restricted condition and provided prepared feed, and hence fish has to spend very meager amount energy in search of food and to escape against enemy attack.

Table 3: Pearson's correlation coefficients' of the different variables of biochemical composition of fish flesh.

Parameters	Moisture	Ash	Protein	Lipid	Carbohydrate	Fiber
Moisture	1					
Ash	-.753**	1				
Protein	-.974**	.831**	1			
Lipid	-.908**	.523*	.876**	1		
Carbohydrate	-.428	.012	.257	.481*	1	
Fiber	.320	.215	-.217	-.444	-.374	1

** Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the level 0.05 (2-tailed).

4. Conclusion

Replacement of plant origin soybean with aquatic animal protein in the form of fish silage; brought significant change in biochemical composition of common carp, hence if such silage incorporated diets will used to feed the fishes, it will help to improve the flesh quality w.r.t. protein content. In the present study, protein content in fish flesh was significantly improved when fish fed with diets replacing 45% (T_4)

soybean meal with fish silage protein, hence it can be considered that for fingerlings of common carp, soybean meal protein can be replaced with fish protein in its silage form upto 45% level.

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