

Effect of Macronutrient Enriched Diet on Hormones and Gonad Maturation of Common Carp & Amur Carp

Mahima Tamta* and R. N. Ram

Department of Fisheries Resource Management, College of Fisheries, G.B.P.U.A.&T., Pantnagar, India

*Corresponding author

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ABSTRACT

The present study was conducted to evaluate the hormones and gonad quality of common carp & amur carp (both male and female) on the basis of varying levels of protein, lipid and carbohydrate incorporated in feed. The study was conducted for 13 months. The observations of present study indicated that for achieving excellent quality of gametes and better performance of reproductive hormones the feed must be incorporated with macronutrients *viz* protein, lipid and carbohydrate in balance. The present study revealed that better gamete quality were attained by the fish group that was fed with high protein and lipid incorporated in feed, in comparison to control diet.

Introduction

Pisces is the largest phylum of living vertebrates, with approximately 30,000 fish species. Fish have occupied almost every aquatic milieu possible on the planet, presenting a massive disparity in temperature, salinity, oxygen, and many other chemical and physical properties of water. These environments have exerted evolutionary pressures on their inhabitants that have resulted in the evolution of the enormous number of fish and an immense variety of reproductive strategies. The physical condition or robustness of fish populations in aquatic ecosystems can be assessed by a variety of means ranging from biochemical and physiological measures to population level a such as growth, reproductive success. Each fish species has evolved in response to a sole set of selective pressures; therefore species differ in their life-history strategies. Each stage of life (i.e., egg, larval,

juvenile, adult) has alternative states. Since each fish species evolves under a unique set of ecological conditions, it has a unique reproductive strategy with special adaptations including anatomical adaptations, developmental adaptations, behavioural adaptations, physiological adaptations, and energetic adaptations. The reproductive process makes species able to perpetuate themselves. The reproductive processes of fish shape the basis for early life. The great variety of these processes among fish makes their study worthwhile, [Breder and Rosen \(1966\)](#). Development and survival of animals greatly depends on environmental conditions, but, along with the environment prevailing during development of offspring, one should also consider the environment to which the parents are exposed. Availability of food in the environment is one of the most important factors affecting both, the parents as well as the progeny. Initiation of reproduction is widely influenced by the

amount of body energy reserves and is responsive to diverse metabolic factors. Ample body energy reserve is crucial for full activation of the hypothalamus–pituitary–gonadal (HPG) axis at puberty and its proper functioning in adulthood [Shahjahan et al., \(2014\)](#). The availability of nutrients in appropriate amount for parents has a strong influence on the health and development of their offspring. Under and over nutrition both can reprogram the development of the next generation.

Dietary sources of energy and nutrients are desirable for growth, reproduction and health. Fish must have all these necessary nutrients and energy in optimum balance and quantity to perform optimally.

Food availability and energy vary with habitats. Animals have the great ability of sensing their inner energy levels and the external energy availability and thus respond accordingly by investing in processes like growth, immune functions or reproduction when food is there in ample amount, or by ensuring their survival when food is scarce. Fish have a firm relationship between food availability and reproduction, since it can alter the timing and duration of spawning, fecundity and egg size [Volkoff et al., \(2009\)](#); [Morgan et al., \(2013\)](#), or the clock of the reproductive cycles. Favourable feeding conditions turn out in early maturation of individuals in contrast a decrease in food availability results a decrease in energy transfer to the gonads.

Nutrition is fundamental to the reproductive performance of all vertebrates, including fish. Successful reproduction requires ample capital in terms of food and energy in order to sustain the high-energy demands for the production of gametes, and other reproductive behaviours, and low food consumption may cause negative energy balance which inhibits the reproductive axis. Less is known about interactions between nutrition and reproduction in fish and a few cases have evaluated the impact of feeds on early and adult reproductive performance.

In general, in fish, studies showed that insufficient food availability or starvation causes gonad regression and a decrease in spawning and in the number of eggs produced by females whereas sufficient food availability promotes better growth and body sizes, causes advance maturation and elevated fecundity, [Volkoff \(2018\)](#). Nutrition is recognized to manipulate gonadal growth and fecundity and it has been generally approved that quality and quantity of feed as well as feeding regime are

essential for spawning and egg quality. [Pezzato et al., \(2005\)](#) postulated that when fishes are kept in an intensive culture, the only source of nutrients is the ration. Therefore, the ration must fulfil the nutritional needs, satisfying the demands for the nutrients necessary for gamete production, and activities of mating or spawning.

Materials and Methods

Experimental Site and its Climate

The experiment was conducted at College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India, geographically located at 29° N latitude, 79.3° longitude and an altitude of 243.3 m above mean sea level (MSL), in Tarai belt of Shivalik range of Himalaya. The experimental site had humid sub-tropical climate characterized by very hot and dry summer and very cold winter.

Experimental Fish Collection

One of the experimental fish (Amur carp) was collected during the month of March 2019 from fish seed hatchery of College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand India and other (Common carp) was collected from a local fish farm. The experimental fish were kept in F.R.P tanks in recirculatory aquaculture laboratory of College of Fisheries, with two replicates of each group and there were two feeding regimes for the control group i.e. control group with fix feeding manner and another one was control group with random feeding manner.

Morphology and Morphometry of Fish

The fish were identified through their morphological characters. The morphometric characters referred to the continuous variables. The morphometry of carp fish was done by using scale for measuring length, and electronic balance for measuring weight.

Statistical analysis

The data obtained from various fish groups was statistically analyzed with design Two- Way ANOVA using Excel sheet.

Results and Discussion

Proximate composition of formulated diets

Results of observed parameters

In the present study the serum estrogen found to be highest in the females of Amur carp that were fed with the lipid diet i.e.154.71 pg/ml, followed by the females of Amur carp with protein diet i.e.153.06 pg/ml. The lowest level of serum estrogen was recorded for the females of common carp with control diet that had random feeding. The highest level of serum testosterone was recorded for the group of male Amur carp that were fed with the lipid diet, and the value was 155.19 pg/ml, followed by the males of Amur carp with protein diet i.e. 153.17 pg/ml. The lowest level of serum testosterone was recorded for the group of male common carp with control diet that were fed twice a day. Water temperature, photoperiod and nutrition status are the main factors which play vital roles in the gonad development (Webb and Doroshov, 2011). Luo *et al.*, (2014) also revealed that the level of E2 and testosterone was elevated in the sturgeon that was fed with the lipid diet (EPA/DHA). Arino (2015) observed the role of lipids and fatty acids in the spermatogenesis of eel (*Anguilla anguilla*), and found the highest level of estrogen & testosterone with lipid diet. Xu *et al.*, (2017) also investigated the effect of lipid sources on gonadal steroidogenesis in the marine teleost, tongue sole (*Cynoglossus semilaevis*) and found a significant increase in the level of estradiol production in females and testosterone production in males. Du *et al.*, (2018) also reported that serum estradiol and testosterone level with high lipid group was significantly recorded higher than those in the other groups of Chinese sturgeon (*Acipenser sinensis*). Higher dietary lipid level promoted gonad development and reproductive performance were also reported in rabbit fish (*Siganus guttatus*) (Duray *et al.*, 1994) & and yellow fin sea bream (*Acanthopagrus latus*) (Zakeri *et al.*, 2009). Narumbuena *et al.*, (2013) also reported an elevation in testosterone hormone in the males of sole (*Solea senegalensis*) fed with levels of lipid.

Gonadal development

It is well accepted that oogenesis, especially vitellogenesis and oocyte maturation in many teleost species, is regulated by ovarian steroids (Fostier *et al.*, 1983; Olivereau and Nagahama, 1983). The gonads are

the end point of the reproductive cascade and perform two main functions, primarily germ cell development (spermatogenesis and oogenesis) and another one is steroidogenesis/growth factor production. Several studies have been demonstrated that reproductive performance and egg quality are being influenced by nutrients like protein, lipid, minerals, vitamins and ration size in fish such as gilthead seabream, *Sparus aurata* (Mourete & Odriozola, 1990), common carp, *Cyprinus carpio* (Manissery *et al.*, 2001). Nutrition is a key factor observed controlling the gonadal development and also fecundity in females. Lipid and fatty acid composition of parent stock diet are the main factors affecting reproduction success and offspring survival (Izquierdo *et al.*, 2001).

In the present study when gonadal development was evaluated, it was observed at some stages of development that some of the males and females of common carp and Amur carp that had fed once in a day in a random manner, had underdeveloped gonads, the term may be coined as gonadal atresia. At this stage, although the gonads of females had gametes but in very few numbers and all were tiny in size. James and Sampath (2004) also observed that the gonad weight increased with time and the increase in feeding frequency in *Betta splendens*.

Fish that received one meal had poor gonadal growth with delayed maturation time, while another group that received two or three meals a day reached sexual maturity earlier with improved gonadal growth. Based on the present study it can be assumed that single feeding frequency in a random manner for long-term (as 13 months in the present study) may be the cause for gonadal atresia since the fish did not receive proper nutrients that are pre-requisite for gonadal growth as well as for gamete formation. Proper nutrition is therefore essential for rearing healthy parent stock and for the production of high-quality gametes and viable offspring as also observed by Izquierdo *et al.*, (2001).

Overall comparison of reproductive performance of common carp and Amur carp

On the basis of the parameters evaluated the present study revealed that Amur carp displayed better performance in both the aspects evaluated. Das (2017) also revealed an enhanced weight gain of Amur common carp over the common carp after a study period of 90 days.

Table.1 Control diet

S. No.	Contents	Percentage %
1	Moisture	17.58
2	Ash	11.96
3	Crude protein	29.20
4	Crude fat	7.89
5	Carbohydrate	33.37

Table.2 Protein diet

S. No.	Contents	Percentage %
1	Moisture	12.90
2	Ash	9.98
3	Crude protein	38.20
4	Crude fat	3.50
5	Carbohydrate	35.50

Table.3 Lipid diet

S. No.	Contents	Percentage %
1	Moisture	18.10
2	Ash	17.60
3	Crude protein	25.29
4	Crude fat	18.26
5	Carbohydrate	20.40

Table.4 Carbohydrate diet

S. No.	Contents	Percentage %
1	Moisture	21.26
2	Ash	12.69
3	Crude protein	28.2
4	Crude fat	5.63
5	Carbohydrate	31.92

Table.5 Serum estrogen of female common carp and Amur carp of Control group

Estrogen (pg/ml)	Control fix		Control random	
	Common	Amur	Common	Amur
March	99.15 ±0.54	99.87±1.1	97.54±2.1	98.58±0.51
April	102.25±4.44	105.56±4.91	78.00±12.4	82.23±11.59
May	102.25±3.30	105.99±5.31	112.25±3.14	112.80±3.83
June	105.25±3.39	96.66±5.25	109.56±2.18	96.21±5.01
July	109.56±0.97	109.59±1.15	88.59±6.47	96.90±8.69
August	115.26±2.84	119.94±2.04	112.26±3.59	123.24±3.21
Sept	105.26±5.42	119.59±6.31	117.45±6.93	119.57±6.76
Oct	109.45±4.45	110.28±7.10	113.54±2.58	102.25±8.57
Nov	119.58±6.82	104.66±3.55	123.56±4.61	129.56±0.65
Dec	111.13±2.08	115.56±2.42	123.79±3.26	128.59±3.12
Jan	198.70±1.30	198.70±2.41	198.70±1.21	198.20±1.23
Feb	209.50±2.12	209.70±4.12	207.80±2.14	207.60±5.12
March	301.20±2.12	309.20±2.13	301.20±2.22	309.20±2.12
Average	137.58	138.86	137.24	138.84
SD	±61.12	±62.72	±62.20	±63.57

Table.6 Serum estrogen of female common carp and Amur carp of Protein group

Estrogen (pg/ml)	Protein	
	Common	Amur
March	86.00±2.23	85.00±2.21
April	87.60±3.21	87.00±1.42
May	90.00±2.23	91.00±2.14
June	107.70±4.12	110.30±2.25
July	111.00±2.25	109.00±3.12
August	121.00±2.52	123.00±3.23
Sept	139.00±2.29	143.50±2.21
Oct	178.00±4.12	178.00±4.12
Nov	179.00±2.61	198.00±1.14
Dec	169.00±2.31	161.00±0.97
Jan	168.80±2.42	172.00±1.26
Feb	198.00±5.21	198.90±1.71
March	317.80±1.24	321.80±2.12
Average	150.22	153.06
SD	±63.67	±64.66

Table.7 Serum estrogen of female common carp and Amur carp of Lipid group

Estrogen (pg/ml)	Lipid	
	Common	Amur
March	81.50±3.79	85.00±3.36
April	88.00±2.41	88.00±1.24
May	91.00±0.93	92.00±1.15
June	98.40±1.14	98.99±4.12
July	109.70±1.26	110.00±1.22
August	112.00±1.42	116.23±3.12
Sept	136.40±2.23	145.00±1.21
Oct	192.80±3.12	197.00±2.12
Nov	198.00±2.13	198.40±2.14
Dec	174.00±2.15	169.00±3.12
Jan	142.60±2.52	153.00±2.12
Feb	197.50±2.62	197.80±3.36
March	319.50±3.21	321.20±2.55
Average	149.33	154.71
SD	±66.88	±63.66

Table.8 Serum estrogen of female common carp and Amur carp of Carbohydrate

Estrogen (pg/ml)	Carb1	
	Common	Amur
March	94.00±2.41	100.00±5.21
April	86.00±2.23	86.90±2.12
May	91.00±1.22	100.19±2.24
June	95.00±1.26	95.55±2.11
July	112.16±1.22	101.39±2.23
August	110.50±1.25	112.00±5.22
Sept	116.50±2.33	116.30±4.55
Oct	178.90±2.44	201.00±4.12
Nov	165.50±2.51	198.00±4.31
Dec	178.40±2.44	178.40±1.55
Jan	184.80±2.61	184.80±4.12
Feb	144.20±2.41	141.55±1.22
March	312.20±2.32	312.60±5.22
Average	143.78	148.36
SD	±62.44	±64.72

Table.9 Serum testosterone of male common carp and Amur carp of Control group

Testosterone (pg/ml)	Control fix		Control random	
	Common	Amur	Common	Amur
March	98.45±2.26	88.28±2.26	88.58±2.81	89.58±2.26
April	84.00±3.12	74.00±2.31	92.56±1.17	98.54±5.26
May	98.99±2.22	98.49±1.14	98.58±4.12	98.29±2.25
June	109.00±2.12	101.00±1.15	110.26±1.22	110.20±5.77
July	110.00±2.26	110.00±1.14	110.00±1.45	110.00±1.12
August	141.00±2.14	147.00±1.12	142.28±5.12	146.59±4.12
Sept	151.00±2.36	151.00±1.47	152.29±1.11	153.47±1.12
Oct	158.00±2.22	169.00±1.37	189.56±1.19	192.81±2.63
Nov	159.00±2.41	178.00±1.52	159.56±2.62	161.40±2.21
Dec	159.00±2.12	169.00±2.36	169.00±2.14	159.00±2.23
Jan	135.00±3.26	152.00±2.52	159.58±2.51	181.25±2.12
Feb	188.90±2.44	198.00±2.29	188.90±2.56	187.80±1.41
March	243.00±5.12	247.00±2.14	247.50±2.52	247.90±4.12
Average	141.18	144.82	146.81	148.98
SD	±98.45	±88.28	±46.50	±46.72

Table.10 Serum testosterone of male common carp and Amur carp of Protein group

Testosterone (pg/ml)	Protein	
	Common	Amur
March	91.00±4.12	82.00±5.22
April	91.00±2.22	91.00±2.12
May	96.00±2.12	96.10±2.23
June	105.00±2.12	107.70±2.23
July	139.00±5.12	141.00±2.62
August	153.00±5.21	162.00±2.74
Sept	161.40±2.14	162.00±4.15
Oct	152.00±4.11	163.00±4.23
Nov	152.00±2.14	167.00±1.44
Dec	139.00±4.12	134.00±1.44
Jan	123.00±5.12	121.90±5.23
Feb	195.00±3.26	196.00±2.15
March	371.00±2.26	364.45±2.23
Average	151.41	153.17
SD	±72.95	±65.37

Table.11 Serum testosterone of male common carp and Amur carp of Lipid group

Testosterone (pg/ml)	Lipid	
	Common	Amur
March	86.00±5.22	72.00±0.99
April	91.00±2.26	100.00±1.21
May	116.70±2.24	110.40±2.31
June	121.80±2.12	111.60±2.22
July	121.00±2.52	134.00±0.77
August	149.40±2.25	151.00±0.15
Sept	150.00±2.12	171.00±1.26
Oct	171.00±2.26	173.00±5.21
Nov	178.00±2.42	164.26±2.23
Dec	159.00±2.26	143.00±2.55
Jan	121.00±2.12	117.00±2.26
Feb	193.90±4.12	189.00±2.23
March	333.33±2.22	371.66±2.12
Average	153.24	155.19
SD	±63.15	±67.30

Table.12 Serum testosterone of male common carp and Amur carp of Carbohydrate group

Testosterone (pg/ml)	Carbohydrate	
	Common	Amur
March	81.00±1.17	79.00±2.23
April	93.40±0.12	101.00±2.11
May	117.77±0.09	107.00±4.15
June	121.00±0.91	112.30±1.12
July	121.00±0.29	121.00±1.11
August	138.80±1.26	154.00±4.12
Sept	151.00±2.15	161.00±4.21
Oct	164.00±1.24	173.00±2.12
Nov	169.00±1.45	164.00±2.12
Dec	143.00±1.78	143.00±2.55
Jan	119.70±1.31	113.00±4.21
Feb	189.80±1.73	198.00±2.26
March	341.50±1.51	345.00±2.55
Average	150.07	151.63
SD	±64.93	±67.16

Figure.1 Serum Estrogen of female Common and Amur carp with Control diet (Fix)

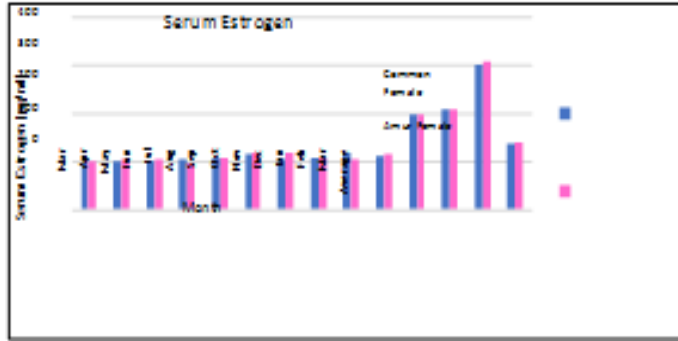


Figure.2 Serum Estrogen of female Common and Amur carp with Control diet (Random)

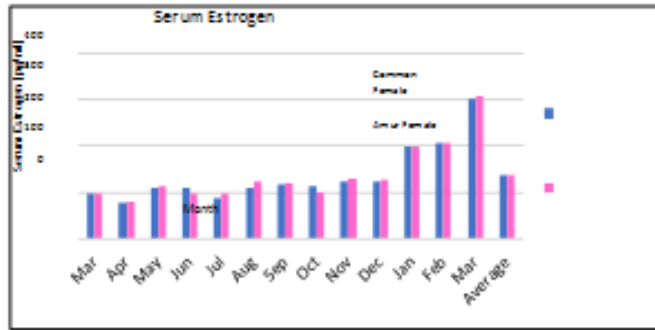


Figure.3 Serum Estrogen of female Common and Amur carp with Protein diet

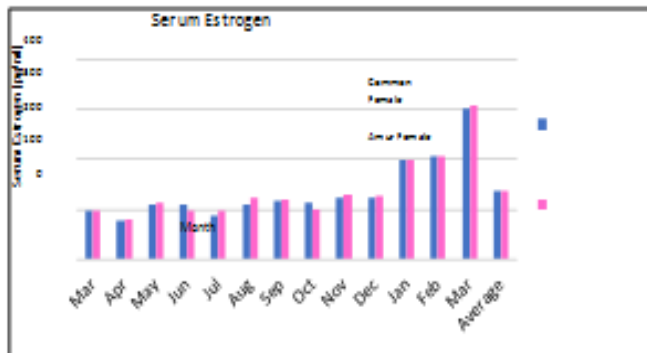


Figure.4 Serum Estrogen of female Common and Amur carp with Lipid diet

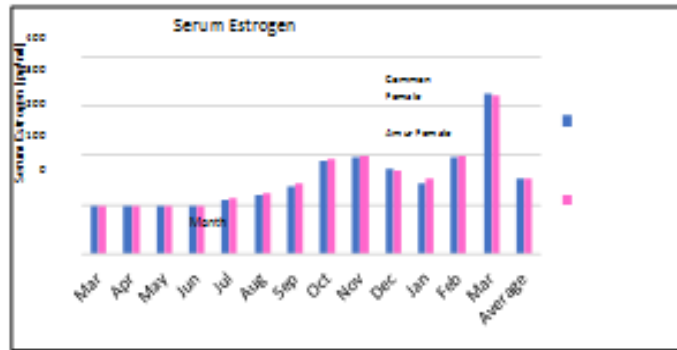


Figure.5 Serum Estrogen of female Common and Amur carp with Carbohydrate diet

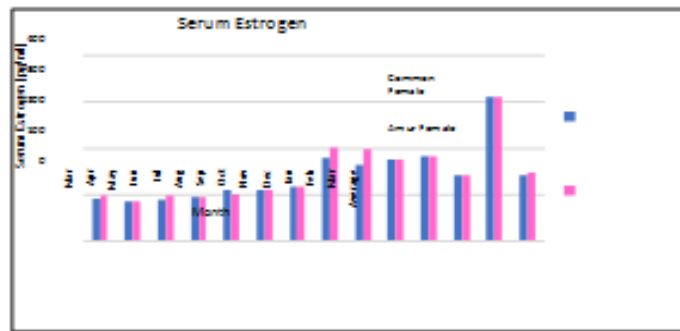


Figure.6 Serum Testosterone of male Common and Amur carp with Control diet (Fix)

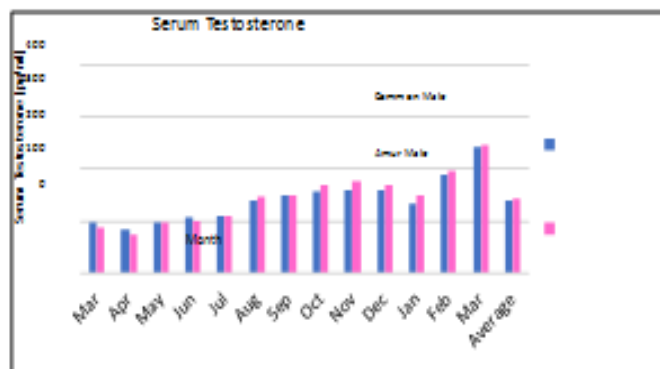


Figure.7 Serum Testosterone of male Common and Amur carp with Control diet (Random)

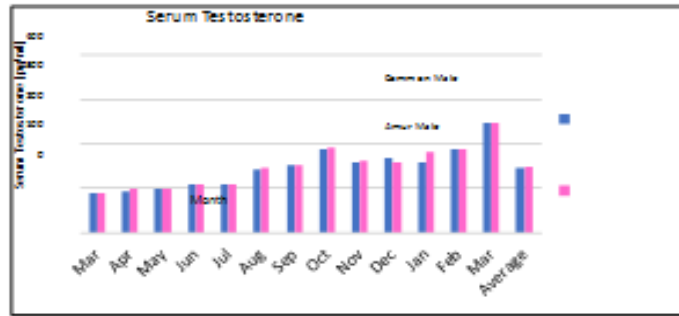


Figure.8 Serum Testosterone of male Common and Amur carp with Protein diet

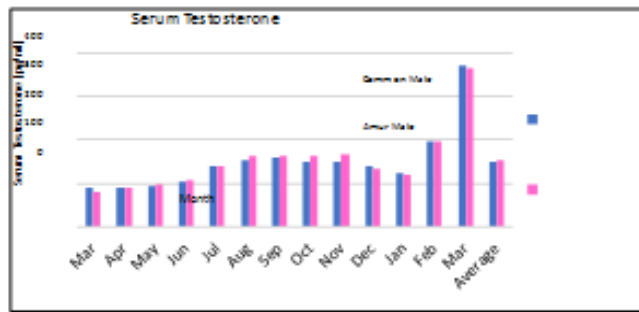


Figure.9 Serum Testosterone of male Common and Amur carp with Lipid diet

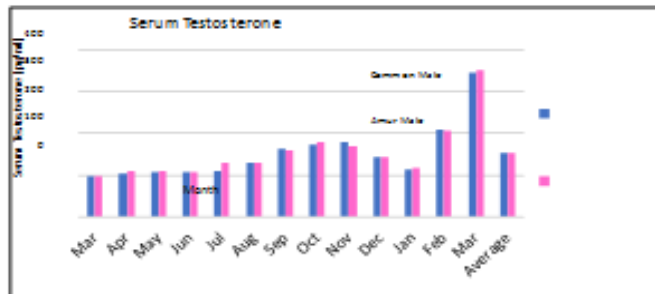


Figure.10 Serum Testosterone of male Common and Amur carp with Carbohydrate diet

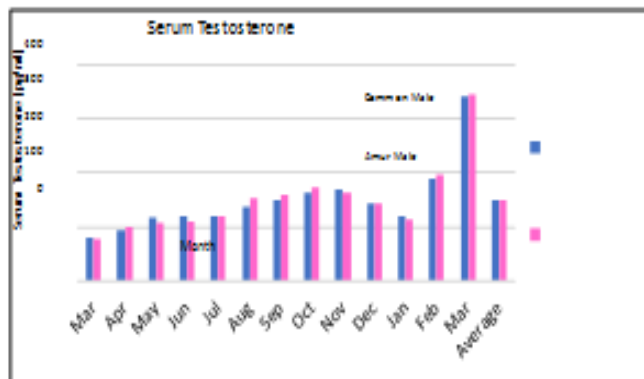
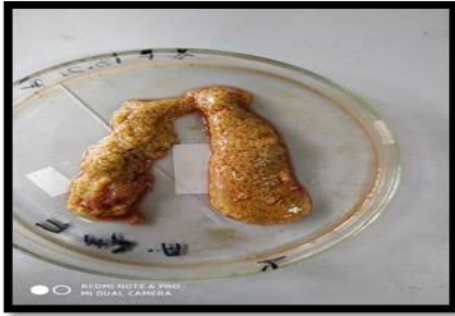


Plate.1 Gonads of female common carp and Amur Carp with different diets



Fully matured gonads of common carp with Protein diet



Maturing gonads of common carp with Control diet (Fix feeding schedule)



Immature gonads of common carp due to restricted feeding with control diet



Gonads of common carp with carbohydrate diet



Fully mature gonads of common carp with Lipid diet



Fully matured gonads of Amur carp with Protein diet ☆



Fully matured gonads of Amur carp



Immature gonads of Amur carp due to restricted feeding with control diet



Control diet (Fix feeding schedule)



Maturing gonads with Carbohydrate diet

Fully matured gonads of Amur Carp with Lipid diet

Plate.2 Gonads of male common carp and Amur carp with different diets



Fully matured gonads of Common carp with Protein diet



Maturing gonads Common carp with Control diet (Fix feeding schedule)



Undeveloped gonads of Common carp due to restricted feeding with control (Random Feeding diet)



Fully matured developed gonads of Common carp with Lipid diet



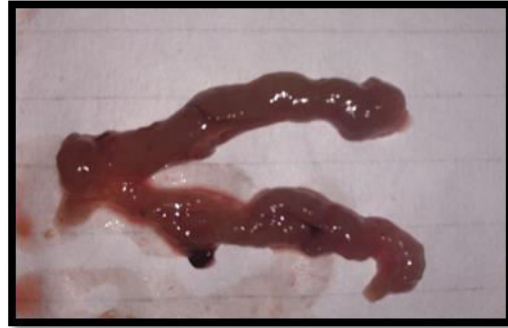
Maturing gonads of Common carp with Carbohydrate diet



Fully matured gonads of Amur carp with Protein diet



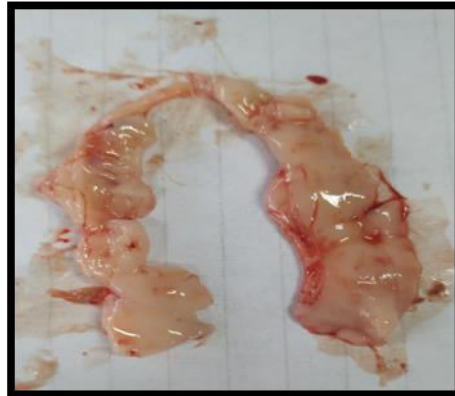
Maturing gonads of Amur carp with Control diet (Fix feeding schedule)



Undeveloped gonads of Amur carp due to restricted feeding with control diet



Fully matured developed gonads of Amur carp with Lipid diet



Maturing gonads of Amur carp with Carbohydrate diet

He also revealed that the egg diameter of Amur common carp was observed to be slightly larger than the local common carp and stated that the information generated through the three year long project strongly suggests adoption of culture of improved variety of the common carp, Amur (Hungarian strain). [Verma and Mandal \(2018\)](#) also found that Amur common carp performed better than other major carps in polyculture system.

The present investigation was carried out to compare the reproductive profile of common carp and Amur carp with special reference to three important constituents of fish feed i.e. protein, lipid and carbohydrate. Evaluating the steroid hormones (estrogen and testosterone) for both male females of candidate species revealed that highest level of estrogen in females and testosterone in males was observed in the lipid diet, and followed by the

protein diet. The highest level of serum estrogen was found in the females of Amur carp fed with the lipid diet i.e. 154.71 pg/ml, followed by the females of Amur carp with protein diet i.e. 153.06 pg/ml. The lowest level of serum estrogen was recorded for the females of common carp with control diet.

The highest level of serum testosterone was recorded for the group of male Amur carp that were fed with the lipid diet, and the value was 155.19 pg/ml, followed by the males of Amur carp with protein diet i.e. 153.17 pg/ml. The lowest level of serum testosterone was recorded for the group of male common carp with control diet. This signifies the importance of protein and lipid in the feed of fish. Gonadal steroids are said and proven to be correlated to the nutritional state of the animal and their deviation appears to be influenced by food intake. The

lipid is the class of macronutrients that is involved in the steroidogenesis and evidenced by many scientists also. The present study also revealed the efficacy of the lipid to enhance the steroid hormones, and their incorporation in the feed can enhance the reproductive performance.

These findings clearly displayed the significance of the major constituents of feed for the growth, well being, and reproductive health of the fish. The feeding frequency along with the quality feed is also important to achieve the overall health of fish. Protein, lipids and carbohydrates are the major constituents that body metabolizes to generate energy for various physiological process.

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Author Contributions

Mahima Tamta: Investigation, formal analysis, writing—original draft. R. N. Ram: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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