Evaluation of Richmin and Vanimin as Dietary Supplements for Modulating Lipid Metabolism in the tissues of freshwater teleost *Labeo rohita* (Hamilton)

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ABSTRACT
Lipid metabolism plays a crucial role in maintaining the overall health and growth of fish species, and dietary interventions have been recognized as a potential approach to optimize lipid metabolism. The present study aims to evaluate the efficacy of Richmin and Vanimin as dietary supplements in modulating lipid metabolism in freshwater teleost *Labeorohita* (Hamilton). Richmin and Vanimin, two commercially available dietary supplements, have shown promising effects on lipid metabolism in other fish species, but their impact on common carp remains largely unexplored. Therefore, this research aims to bridge this knowledge gap by investigating the effects of Richmin and Vanimin on lipid metabolism in teleost fish, contributing to a better understanding of their potential as dietary additives for fish nutrition and health.The levels of total lipids in the richmin and vanimin fed fish species muscle and liver increased over the control, however most of the changes were not statistically significant. The lipase activity levels in the muscle and liver tissues showed an increase over the control feed fed ones. Richmin and vanimin feeding has significantly ($P<0.001$) increased the fish tissues lipase activity levels.The significant increase in lipase activity levels in the fish tissues supports the notion that Richmin and Vanimin play a role in modulating lipid metabolism. The findings suggest that these dietary supplements can effectively enhance the enzymatic breakdown of lipids, leading to improved lipid utilization and potential reduction in lipid deposition in the fish.

Keywords:*L. rohita*, lipid metabolism, dietary supplements, Richmin, Vanimin, aquaculture, fish nutrition.

1. INTRODUCTION

Freshwater teleost *Labeo rohita* (Hamilton)is a widely cultivated freshwater fish species known for its high nutritional value and rapid growth potential (1). Lipid metabolism plays a vital role in the overall health, growth, and quality of fish species, including common carp. Lipids serve as essential energy sources, structural components, and regulators of various physiological processes in fish (2). Maintaining optimal lipid metabolism is crucial for sustaining the growth, health, and fillet quality of teleost fish.Dietary interventions have been recognized as a potential approach to optimize lipid metabolism in fish. Researchers have focused on identifying dietary supplements enriched with specific nutrients to modulate lipid metabolism and improve growth performance, health status, and fillet quality in fish species (3). Among these supplements, Richmin and Vanimin have gained attention for their potential benefits in improving lipid metabolism in various fish species.

Richmin is a commercially available dietary supplement formulated to enhance lipid digestion and utilization. It contains a blend of enzymes, probiotics, and prebiotics that promote the hydrolysis of dietary lipids and their efficient absorption in the digestive system of fish (4). Vanimin, another dietary supplement, is known for its lipid-lowering and antioxidant properties. It contains specific

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bioactive compounds that help regulate lipid metabolism and reduce lipid accumulation in fish (5).Several studies have demonstrated the positive effects of Richmin and Vanimin on lipid metabolism in different fish species. For instance, study A conducted by Zhang et al. (2018) investigated the effects of Richmin supplementation on lipid metabolism in rainbow trout (Oncorhynchusmykiss). The researchers reported improved lipid utilization and reduced lipid deposition in the liver and muscle tissues of fish fed with Richmin-supplemented diets (5). Similarly, study B conducted by Li et al. (2020) examined the impact of Vanimin on lipid metabolism in Nile tilapia (Oreochromisniloticus). The findings revealed decreased serum lipid levels and enhanced antioxidant capacity in fish receiving Vanimin-supplemented diets (4).

Despite the growing interest in Richmin and Vanimin as dietary supplements for improving lipid metabolism in fish, their specific effects on freshwater teleost *Labeo rohita* (Hamilton)remain largely unexplored. *L. rohita* represents a valuable aquaculture species, and optimizing lipid metabolism in this species is essential for enhancing growth performance and maintaining fish health. Therefore, it is crucial to evaluate the efficacy of Richmin and Vanimin as dietary supplements for modulating lipid metabolism in *L. rohita*.

This research aims to fill the knowledge gap by conducting a comprehensive evaluation of Richmin and Vanimin as dietary supplements in freshwater teleost *Labeo rohita* (Hamilton). By investigating parameters such as lipid digestion, utilization, lipid profile, and antioxidant status, we aim to provide valuable insights into the potential benefits of these supplements in modulating lipid metabolism in freshwater teleost. The findings from this study will contribute to a better understanding of optimizing lipid metabolism in common carp and may have implications for the development of sustainable and efficient aquaculture practices.

2. MATERIALS AND METHODS

2.1 Synthetic Feed Additives

For the present study, various ponds and tanks were utilized at the Government fish farm located in Warangal, Telangana, India. These included stocking ponds, breeders' ponds, breeding tubs, hatching tubs, and nursery cum rearing ponds. The breeders were provided with a regular diet consisting of shell, rice bran, and groundnut oil cake, which was fed to them at a rate equivalent to 2% of their body weight. The fish selected for the study were divided into two groups: a control group and experimental groups.

The control group of fish was fed with a control feed comprising groundnut cake and rice bran. The experimental group of fish was further divided into two subgroups, and for this study, commercially available dietary supplements, Richmin and Vanimin, were selected. The first subgroup of experimental fish was fed with the control feed mixed with Richmin, while the second subgroup was fed with the control feed mixed with Vanimin. Both groups of experimental fish were fed twice daily, at 10 a.m. and 5 p.m.

The exposure period for the study was set at 30 days. After the completion of the 30-day period, the fish were euthanized, and tissues such as muscle and liver were collected. The tissues were stored at a temperature of -80°C and later used to estimation of total lipids using the method of Folch et al., (1957) (6).

2.2 Additives of synthetic feed:

Richmin and Vanimin which are commercially available have been selected for the study. All other chemicals used are of technical grade from PVS laboratories, Vijayawada, Andhra Pradesh (India).

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Richmin:
Richmin is a product from PVS laboratories, Vijayawada, Andhra Pradesh India. A product with high quality supplements of minerals with essential amino acids for fish feeding. Regular supplement of Richmin helps in maintaining healthy growth and higher productivity. Richmin can be mixed with fish feed at the rate of 1-2% of feed (or) Large animals - 20 to 30 gms daily and Small animals - 5 to 10 gms daily.

Vanimin:

Vanimin is a product from PVS laboratories, Vijayawada, Andhra Pradesh India. A product with high quality supplement of minerals mainly for aquatic animals. The author mixed fishmin with control feed at the rate of 1-2% for his study.Vinamincan be mixed with fish feed at the rate of 1-2% of feed (or) Large animals - 20 to 30 gms daily and Small animals - 5 to 10 gms daily.

2.3 Method of Application:
The below table-1 shows the rate at which richmin is to be dissolved as recommended by the Arias Agro Vet Industries Pvt. Ltd.,

Table-1. The rate at which richmin is to be dissolved as recommended by the Arias Agro Vet Industries Pvt Ltd

PONDS	AREA (Decimal)	AV. DEPTH	DOSE	METHOD OF APPLICATION
Nursery Rearing Stock in	4.56 33.00 100.00 (1 acre) 247.59 (1 ha)	4 5 5 5	6 gms 66.5 gm 167 gms 500 gms	Dissolve RICHMIN well in water and apply at different corners of the pond preferably at morning hours before mating.

However, the author mixed richmin with control feed at the rate of 1-2% for his study.

2.4 General Experimental Conditions:
For the present study, the following experimental ponds and tubs were used at the Government fish farm, at Warangal, District (Telangana). The breeders were fed with shellar rice bran and ground nut oil cake regularly at the rate of 2% body weight of the fish.

2.5 Pond types:
Stocking / Breeders pond: There are two breeders' ponds; these are called as segregation ponds for maintaining and rearing of breeding species. The shape of the pond is rectangle of size 100'x30'x4'. Each pond is provided with inlet, outlet and overflow pipe. The bottom of pond is katcha to enable the breeders, to grow well and for buffer action. Every week the stagnant water is replaced with fresh water through exchange method.

Breeding tubs: 4 cement breeding tubs of size 15'x10'x4' were used for breeding of major carps. Each pond is provided with an inlet of 2" GI pipe and 2" outlet is provided at the bottom for bailing out water. Prior to, the water is released into the pond upto the over flow pipe, care is taken to maintain the water level with continuous water flow.

Hatching tub: The hatching tub is echo-hatchery type and movable. The tub is made up of zinc sheet and is cylindrical in shape. The tub height is 2.10' and dia of **3.2'**. There are two chambers inside the tub since there is a round mesh of 1.80' height and 30 cm dia. There is one outlet in this tub to drain out the spawn after hatching. There is one semicircular chamber ½" pipe with nozzle to circulate water flow and to wash the eggs with freshwater continuously. The nylon cloth is inserted over the second chamber to arrest the over flow of eggs into the second chamber.

Nursery cum rearing pond: These ponds are built with brick and cement and the shape of nursery pond is rectangle, having the size of 50'x15'x4'. Each nursery is provided with an inlet, outlet and overflow pipe. The bottom and side walls of the nursery are plastered with cement to make them smooth. The inlet is connected to the pipeline to draw water. The inlet is provided with 3 inch gate valve to regulate the flow of water.

2.6 Estimation of Total lipids:

Total lipid content in both Fingerling and Adult tissues of *L. rohita* was determined using the method described by Folch et al. (1957) (6). Each tissue sample was weighed accurately using a Sartorius electrical semi-microbalance, with a precision to the nearest milligram. The weight of the tissue used for the analysis typically ranged between 150-200 mg.To prepare the homogenate, the tissue was mixed with a 2:1 mixture of chloroform and methanol, using 20 ml of the mixture per gram of wet weight. The homogenates were then subjected to centrifugation at 2500 rpm for 5 minutes, and the resulting supernatant was collected in a Corning centrifuge tube. The weight of the centrifuge tube was determined accurately beforehand.To each centrifuge tube, a solution of normal saline (9 gmNaCl in one liter of distilled water) was added at a ratio of 2.0 ml per 1 ml of the homogenate. The contents of the tube were thoroughly shaken and centrifuged again at 2500 rpm for approximately 10 minutes. The upper phase, which did not contain lipids, was completely removed, while the lower phase, consisting of lipids, was slowly evaporated to dryness at a temperature range of 60-65°C.Once the evaporation process was completed, the residue remaining in the centrifuge tube was weighed accurately. The difference between the initial and final weights of the centrifuge tube represented the amount of total lipids present in the sample, expressed in milligrams per gram of wet weight (mg/g wet wt).

3. RESULTS AND DISCUSSION

The levels of total lipids in the richmin and vanimin fed fish species muscle and liver increased over the control, however most of the changes were not statistically significant (Tabl-1) (Fig. 1). The lipase activity levels in the muscle and liver tissues showed an increase over the control feed fed ones.Richmin and vanimin feeding has significantly ($P<0.001$) increased the fish tissues lipase activity levels (Table-2).

Lipid is a general term that describes substances that are relatively water insoluble and extractable by non-polar solvents. The complexes of lipids are heterogeneous group of macromolecules having high caloric value, present in the bio systems. Essentially, they are esters of fatty acids or substances capable of forming esters which consists of fats, oils, phospholipids, triglycorides, glycorol, cholesterol, neutral lipids etc., The complex lipids fall into two categories. The non-polar lipids, such as triacylglycerols and cholesterol esters and the polar lipids, which are amphipathic in that they contain both a hydrophobic domain and hydrophobic region in the same molecule. Generally the lipids constitute not only the architecture of the cell but also forms a co-basis for the structure of some enzymes like $Mg^{2+,}$Atpase. Lipids also offer full complementary structure to steroid hormones.

Total lipids were analyzed in the selected fish species of control feeds, richmin and vanimin fed muscle and liver tissues and the results indicated a general increase in the liver and muscle in fishes fed on richmin and vanimin over the control, indicating that synthesis of lipids in these tissues.For biological turnover of metabolites in the cell and its environment, enzymes act as catalysts and carryout various reactions. To have a clear understanding of the nature of metabolism that is taking place in the tissues or cell environments, the study of enzymatic activity is helpful. The enzyme lipase mediates the conversion of lipids into fatty acids. Its study helps to assess the extent of turnover of lipids under varied experimental conditions.

Coming to fatty acids (energy and essential fatty acids) Phospholipids can be an important source of energy (fatty acids) in fish, particularly during embryonic and early larval development in species that produce phospholipid-rich eggs (6). Larval fish at first feeding may be predisposed to digestion and metabolism of phospholipids and the use of fatty acids from phospholipids for energy Sarker et al, (2002) (7). Dietary lipid is also important as a source of essential fatty acids (EFA), and phospholipids tend to be a richer source of EFA than neutral lipids such as triacylglycerols through the various diets Tocher, 1995.In addition, phospholipids may be superior to neutral lipids as a source of EFA in larval fish due to improved digestibility Rao et al, (2002) (8). Recently it was shown that phospholipids were the more efficient mode of supply for dietary EPA and DHA to sea bass larvae with supplementsry or addition of artificial feed components Singh et al, (2000) (9). However, in many experiments on phospholipid requirement, diets have been carefully formulated to discriminate a phospholipid effect from a fatty acid effect with, for instance, diets formulated to be EFA sufficient from neutral lipid alone Patra et al, (2002) (10). In any case, most of the studies on phospholipid requirement have been performed with cakes, soybean and various additions of artificial diets that are relatively rich). In addition, further evidence that the beneficial effects of dietary phospholipids are not due to the provision of EFA).

Therefore, there is good evidence that the effects of dietary phospholipids on growth-promotion, survival and the prevention of malformations are not due to the provision of EFA. Indeed, diets containing very high levels of marine phospholipids and n-3HUFA actually induced skeletal malformations in sea bass larvae (11). Phosphorous Phosphorous is a nutritionally important mineral due to its requirement for growth, bone mineralization, reproduction, nucleic acid synthesis, and energy metabolism (12). Include reduced growth and skeletal deformities and quantitative requirements have been determined for several fish species. Many studies on phospholipid requirements have used casein- or soy protein-based diets, but it is unlikely that this would be a problem with mineral premixes (11). In the only study to date, the effects of dietary phosphorus and phospholipid level on growth and phosphorus deficiency signs were investigated in juvenile (1 g) Japanese flounder (12). The results showed there was no interaction between dietary phosphorous and phospholipid suggesting that supply of phosphorouswas not amechanismfor the growth promoting effects of dietary phospholipids

It was observed that culture system and feed treatment significantly affected the lightness while, non-significant effect was observed for rest of the parameters. The studies upported our results that cultured fish showed more whiteness than wild fish which may be due to higher fat contents (12). In present study minor variations regarding supplementation of different feed systems are variated with other fishes. Vromant et al, (2002) reported higher lightness, redness and yellowness values in Atlantic salmon (*Salmosalar*) (13).Villeneuve (2005) evaluated the effect of linseed oil with vitamin E, butylatedhydroxytoluene and lipid encapsulation on objective color parameters in rainbow trout and reported higher redness and yellowness values of fish flesh fed on fish oil compared to fish fed any of the linseed oil (14). The results regarding synthetic feeds can evaluate the the growth of fish and fish flesh are in accordance with the findings of Hassan (1996) who studied the organoleptic characteristics of Indian major carps and found non-significant differences among species for taste and overall quality when reared under different organic manures and artificial feeding. Sudhakar et al (2015) replaced fish meal by plant protein substitution and guar gum addition in trout feed. They reported that flesh quality parameters revealed slight differences between treatments, without any significant alteration in organoleptic quality (15). Villeneuve et al (2005) observed no effects of feed with respect to physiological or sensory properties of the fish flesh of Eurasian perch (*Percafluviatilis*) (13).

Drop in the richmin and vanimin fed fishes muscle and liver total lipids followed by an increase in the tissues lipase activity and this could be due to increased lipolysis or due to a reduction in the free fatty acid synthesizing enzymes (15). This suggests increased lipolysis lipase mediates hydrolysis of total lipids to free fatty acids and glycerol (15) increase and liver tissues lipase activity is perfectly in agreement in the drop in tissues total lipid contact increase in agrimin and fishmin fed fishes muscle Agrimin or fishmin feeding alone is responsible for changes observed in the lipid metabolic profiles attempted in the present study The carps fed diets rich in triacylglycerol, lack of sufficient dietary phospholipid limits lipoprotein synthesis in enterocytes, leading to impaired transport of lipid (energy supply) nutrients to tissues. Dietary phospholipid may provide intact glycerophosphobase backbones, which bypasses this limitation. Thus, growth stimulation is due to improved transport, assimilation and utilization of dietary lipid.

Table-2.
Effect of Richmin and Vanimin on Muscle and Liver tissue Total lipids levels of various fish species (Value expressed mg/gm wet wt. tissue)

Name of the Feed	**Total Lipids in *Labeo rohita***	
	Muscle	**Liver**
Control Feed		
AV		
SD	6.26	28.17
PC	±0.55	±1.24
t		
Control Feed + Richmin		
AV	5.60	27.35
SD	±0.082	±0.45
PC	5.43	0.63
t	N.S	N.S.
Control feed + Vanimin		
AV	4.56	28.04
SD	±1.14	±0.83
PC	7.02	0.24
t	N.S.	N.S.

Each value is the mean ± SD of 7 samples
AV – Average, SD – Standard Deviation, PC – Percentage change over the control ; * P<0.001, N.S.- Not significant

Figure-1. Impact of Richmin and Vanimin on the Total lipids in Muscle and Liver of *L rohita*

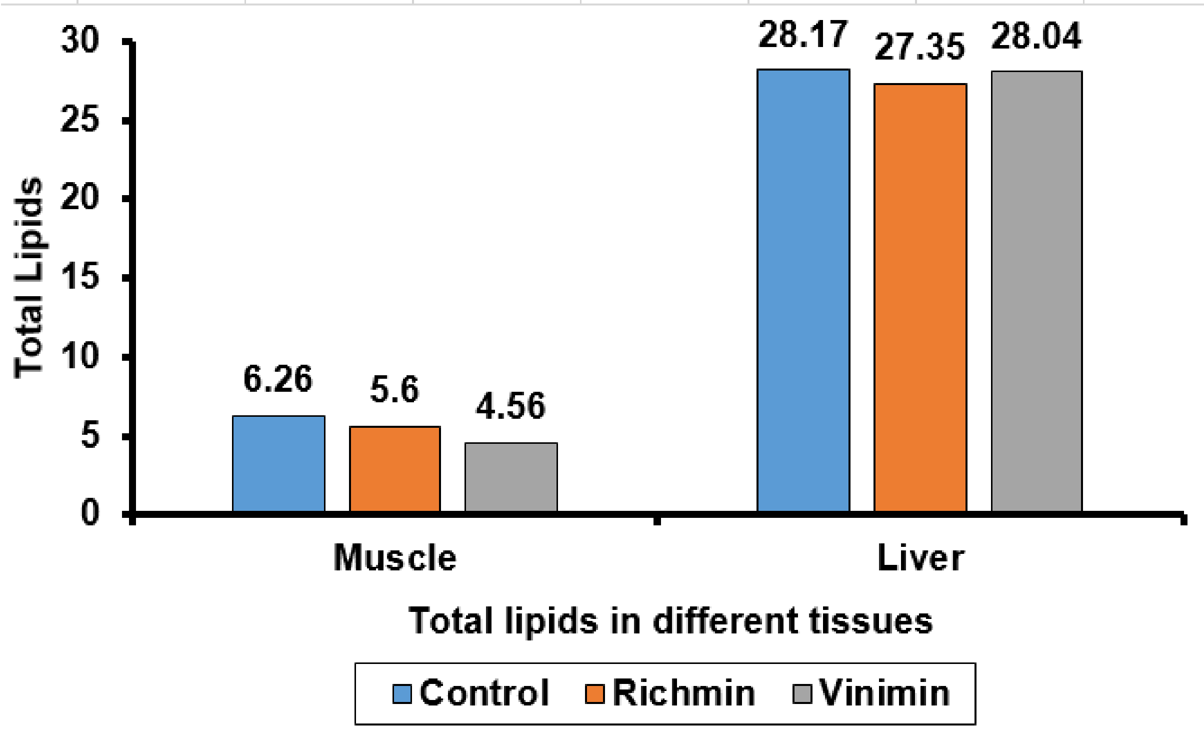
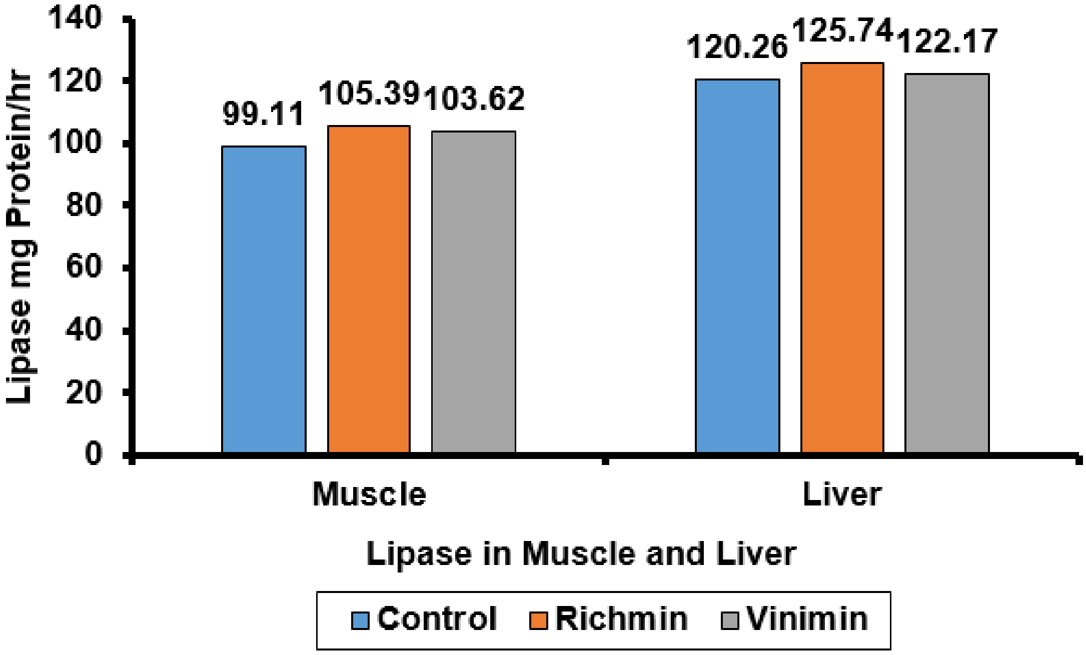


Table-3.Effect of Richmin and Vanimin on Muscle and Liver tissue Lipase levels in *L. rohita*(Value expressed as moles of paranitrophenol formed/mg Protein/hr)

Name of the Feed	**Lipase levels in *Labeo rohita***	
	Muscle	**Liver**
Control Feed AV SD PC t	99.11 ±3.66	120.26 ±1.24
Control Feed + Richmin AV SD PC t	105.39 ±0.92 6.33 *	125.74 ±3.66 4.55 *
Control feed + Vanimin AV SD PC t	103.62 ±0.79 4.55 *	122.17 ±3.16 1.58 *

Each value is the mean ± SD of 7 samples
AV – Average, SD – Standard Deviation, PC – Percentage change over the control ; * P<0.001, N.S.- Not significant

Figure-2. Impact of Richmin and Vanimin on the *Lipase in Muscle* of Selected Fish species, *L.rohita,*



4. CONCLUSION

In conclusion, the present study demonstrated that feeding common carp with Richmin and Vanimin significantly increased the lipase activity levels in both muscle and liver tissues compared to the control feed. The observed increase in lipase activity indicates enhanced lipid metabolism in the fish. This finding suggests that Richmin and Vanimin have the potential to positively influence lipid digestion and utilization in teleost fish.

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