UTILIZATION OF DETOXIFIED *JATROPHA* KERNEL CAKE IN THE DIET OF *CLARIAS MAGUR* (HAMILTON, 1822) FINGERLINGS: FOR GROWTH PERFORMANCE

Dissertation submitted in partial fulfillment

of the requirements

for the degree of

M. F. Sc. (AQUACULTURE)

by

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DECLARATION

I hereby declare that the dissertation entitled, **"Utilization of detoxified *Jatropha* kernel cake in the diet of *Clarias magur* (Hamilton, 1822) fingerlings: for growth performance**," is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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(Dedicated to my Baba And Maa)

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CERTIFICATE

Certified that the dissertation entitled **"UTILIZATION OF DETOXIFIED JATROPHA KERNEL CAKE IN THE DIET OF *CLARIAS MAGUR* (HAMILTON, 1822) FINGERLINGS: FOR GROWTH PERFORMANCE"** is a bonafide record of independent research work carried out by **Ms. PUJA CHAKRABORTY** during the period of study from August, 2017 to June, 2018 under our supervision and guidance for the degree of **Master of Fisheries Science (Aquaculture)** and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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ABSTRACT

A 60 days feeding trial was conducted to study the effect of detoxified *Jatropha* kernel meal on nutrient utilization, growth performance and physio-metabolic response of *Clarias magur* fingerlings. Four iso-nitrogenous diets were prepared with different inclusion levels of detoxified *Jatropha* kernel meal (DJKM) *i.e.,* Control (0%), T1 (10%), T2 (20%) and T3 (30%). 240 numbers of *C. magur* (magur) fingerlings were randomly distributed in four different experimental groups with three replicates. Each replicate contains 20 fishes with an average weight of 5.0 ± 0.5 g and fed till satiation, daily twice for 60 days. A significant difference ($P<0.05$) was observed in weight gain %, specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER). 10% DJKM fed group (T1) and control group showed higher weight gain %, SGR, FER and PER with the values of 107.51 ± 3.02, 1.21 ± 0.02, 0.41 ± 0.004 and 0.43 ± 0.001 respectively and lower values were observed in T2 and T3 fed groups. Similarly, digestive enzymes such as Amylase, Protease, Lipase activity were also significantly higher in the fishes reared in Control and T1 fed groups. Study of metabolic enzyme revealed that metabolic enzyme activity (AST, ALT & ALP) decreases with the gradual increase in the inclusion level of DJKM whereas, SOD and Catalase were showing higher activity with the increased inclusion level of detoxified *Jatropha* kernel meal in the diet. Hence, it is found that inclusion of DJKM upto 10% in the diet of *C. magur* fingerlings showed better nutrient utilization and growth performance.

सारांश

पोषक उपयोग, विकास प्रदर्शन और क्लारिया मैगुर फिंगरलिंग के भौतिक-चयापचय प्रतिक्रिया पर डिटोक्सिफाइड जेट्रोफा कर्नेल भोजन के प्रभाव का अध्ययन करने के लिए 60 दिनों का भोजन परीक्षण आयोजित किया गया था। चार आईएसओ-नाइट्रोजेनस आहार डिटॉक्सिफाइड जेट्रोफा कर्नेल भोजन (डीजेकेएम) यानी नियंत्रण (0%), टी 1 (10%), टी 2 (20%) और टी 3 (30%) के विभिन्न समावेशन स्तरों के साथ तैयार किए गए थे। सी। मगुर (मगुर) के 240 नंबरों को तीन प्रतिकृतियों के साथ चार अलग-अलग प्रयोगात्मक समूहों में यादच्छिक रूप से वितरित किया गया था। प्रत्येक प्रतिलिपि में 5.0 ± 0.5 ग्राम के औसत वजन के साथ 20 मछलियों होते हैं और संतृप्ति तक खिलाया जाता है, दैनिक 60 दिनों के लिए दो बार। वजन वृद्धि%, विशिष्ट वृद्धि दर (एसजीआर), फ़ीड रूपांतरण अनुपात (एफसीआर), फ़ीड दक्षता अनुपात (एफईआर) और प्रोटीन दक्षता अनुपात (PER) में एक महत्वपूर्ण अंतर (पी <0.05) मनाया गया था। 10% डीजेकेएम फेड ग्रुप (टी 1) और कंट्रोल ग्रुप ने क्रमश: 107.51 ± 3.02, 1.21 ± 0.02, 0.41 ± 0.004 और 0.43 ± 0.001 के मूल्यों के साथ उच्च वजन लाभ%, एसजीआर, एफईआर और PER दिखाया और टी 2 में कम मूल्यों को देखा गया और टी 3 खिलाया समूह। इसी प्रकार, पावर एंजाइम जैसे कि एमिलेज़, प्रोटेज़, लिपेज गतिविधि नियंत्रण और टी 1 फेड समूहों में रखी मछलियों में भी काफी अधिक थी। चयापचय एंजाइम का अध्ययन से पता चला है कि चयापचय एंजाइम गतिविधि (AST, ALT ALP) DJKM के समावेशी स्तर में क्रमिक वृद्धि के साथ कम हो जाती है, जबकि, वतन और Catalase detoxified जटरोफा की वृद्धि का समावेश स्तर के साथ उच्च गतिविधि दिखा रहे थे आहार में गिरी भोजन । इसलिए, यह पाया गया है कि मग्घर fingerlings के आहार में 10% तक DJKM का समावेश बेहतर पोषक तत्व उपयोग और विकास के प्रदर्शन को दिखाया ।

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. INTRODUCTION

The worlds' population is expected to reach 9.7 billion by 2050, and the global demand for protein rich feed ingredients, for the production of animal protein, is increasing gradually. Though, aquaculture production continues to grow at a relatively high rate compared to other animal production sectors, it is not enough to meet the demand due to ingredients shortage, mainly caused by the high demand and low supply of fish meal and soybean meal. Presently, the global production of commercial aquafeeds stand at about 34.4 million tons (Alltech, 2013), and is expected to reach 71 million tons by 2020 (FAO, 2012). This will require a huge amount of conventional protein feed resources, most of which are currently becoming scarce, competitive and unsustainable. Aside from the limited availability, the price of the commonly used ingredients in the aqua feed is on the rise due to competition with other animal feed sectors. This has forced the researchers to explore for an alternative protein source that is nutritionally compatible for cultured species, and cost efficient for sustainable aqua feed development.

Aquaculture in India has attained a status of fast expanding industry with the bulk of its production about 87 percent contributing from the indigenous and exotic carps. The annual fisheries and aquaculture production of India has increased from 0.75 million tonnes in 1950-51 to 9.6 million tonnes in 2013-2014. Globally India is in the second position, after China, with respect to annual fisheries and aquaculture production (excluding seaweed).

In India, freshwater aquaculture production comprises about 2.36 million ha of ponds and tanks which accounts for almost 55% of the total fish production. Additionally, freshwater aquaculture also includes lakes, irrigation canals, reservoirs and paddy fields. Indian major carps namely catla (*Catla catla)*, rohu (*Labeo* reohita) and mrigal (*Cirrhinus mrigala)* make upto 70% to 75% of the total freshwater fish production, where as silver carp, grass carp, common carp and catfish contribute 25% to 30% of the total production.

Due to a gradual decline in marine fish catch, people started to give more emphasis on aquaculture and for the diversification of species. It has been observed that more priority has given to the commercial farming and production of catfishes due to their high market demand and suitability in the culture system. It is the boon of a huge potential resource in the country in the form of many inland water resources.

Among the indigenous catfishes, air-breathing catfish such as *Clarias magur*, *Heteropneustes fossilis* and *Aorichthys seenghala etc.* have been prioritized for aquaculture and conservation purpose. The culture of magur has good prospects in developing domestic trade, due to its high market price, medicinal value, better palatability and rich protein content. The fish is highly demanded in the North Eastern part of India mainly in Assam, West Bengal, Bihar and Odisha for its high nutritional value as it contains a higher percentage of protein and iron as compared to other freshwater edible fish species (Sakhare and Chalak, 2014). Its fat content is very low and therefore it is easily digestible.

As an omnivorous fish, magur requires a considerable amount of crude protein in its diet. Formulating the feed with high protein content is the most expensive factor in aquaculture production, since the protein component in fish diet escalates the feed cost. Traditionally, fish meal (FM) is the main source of dietary protein for fish. In recent years, its increasing cost, limited availability in the market and poor quality had stimulated several studies on its partial or complete substitution with alternative protein sources (Kaushik *et al.,* 1995; Fournier *et al.,* 2004).

Hence, the plant-based protein sources are used in catfish feeds and commonly used feedstuffs are soybean meal, corn grain, cottonseed meal, corn gluten feed, corn germ meal, distillers dried grains with solubles, wheat middlings, and rice bran. Apart from soybean meal, other plant-based ingredients are deficient in lysine but supplemental lysine can cope up this situation. Some of the plant proteins contain toxins and anti-nutritional factors, which are either present in very low concentrations or can be detoxified during processing or feed manufacturing. In catfish feed, soybean meal is incorporated at the rate of 40–50% because of its high protein (48%) content and best amino acid profile compared to other common

plant feedstuffs, and due to its high digestibility and palatability to catfish. According to FAO (2008), a notable increase of 60% in the price of soybean meal has been observed. However, the dramatic increase in the price of soybean meal, initiated a search for the alternative feedstuffs to replace the soybean meal without affecting fish growth. Many studies indicate that cottonseed meal, corn germ meal or a combination of cottonseed meal and corn gluten feed, or distillers dried grains with soluble along with supplemental lysine can be used as a replacement of soybean meal.

The *Jatropha curcas* (physic nut) is one such plant that can be used as an alternative source of protein. It is known as a hardy plant, thrives generally on the degraded land area and it requires a very less amount of nutrients and water. The seeds have been extensively investigated as a source of oil. The defatted *Jatropha* kernel meal (DJKM) is an excellent source of protein (603–624 g kg^{-1} DM) (Makkar and Becker, 2009). However, the presence of high amounts of anti-nutritional factors (ANFs) such as phytate, trypsin inhibitor, and lectin (Makkar *et al.,* 1998) and the major toxic components phorbol esters (PEs) (Makkar and Becker, 1997) restrict their use in fish feed, Hence it needs to be detoxified before its usage.

With this background, the present experiment was mainly done to evaluate the growth and physio-metabolic response of *Clarias magur* under different inclusion level of Detoxified *Jatropha* kernel meal (DJKM) in its diet with the following objectives:

OBJECTIVES

1. To study the effect of detoxified *Jatropha* kernel cake on nutrient utilization and growth performance of *Clarias magur*.

2. To study the effect of detoxified *Jatropha* kernel cake on physio-metabolic responses of *Clarias magur*.

2. REVIEW OF LITERATURE

2.1. Biology and nutritional requirement of *Clarias* species

In many Asian and African countries catfishes of the genus *Clarias* are mostly of commercial importance. The farming of *Clarias magur* mainly done in Indonesia, Thailand and India; *C. fuscus* in Philippines; *C. macrocephalus* in Philippines, Thailand, and Malaysia. *Clarias gariepinus* mostly done in Africa (mainly Nigeria and South Africa) and Europe (Germany, Netherlands, Belgium) (Verreth *et al*., 1993). *Clarias magur*, also known as walking catfish, is widely found throughout the Indian sub-continent. Due to its high growth rate and marketability, it is mostly cultured either as a single species or along with other catfish species (Thakur, 1991). Nutritional information on this species is limited to its protein content (Chuapoehuk, 1987; Khan and Jafri, 1990). For larval rearing of *Clarias magur* and *Clarias gariepinus***,** both extensive and intensive methods have been successfully developed (Knud- Hansen *et al*., 1990).The larval rearing strategies comprise of an "early" and an "advanced" larval stage, which requires a specific feeding regime. During the early stage, *i.e.,* the first two weeks after they start exogenous feeding, a larval diet consists of mainly live food is needed. After this early stage, *i.e.,* in the "advanced" larval stage the larvae are less dependent on live food, and administration of formulated diet is quite common during this period. Weaning to the "adult-like" dough diet is completed within 20 days after they start external feeding. For the African species *C. gariepinus***,** weaning starts after 10 – 12 days (Verreth and Bieman, 1987; Polling *et al*., 1988). Before that period, a particular larval diet is required, either in the live food form (Polling *et al*., 1988) or a Single Cell Protein-based diet form (Uys and Hecht, 1985). Yasmin *et al.* (1998) reported that C. *magur* larvae could be successfully reared with *Tubifex sp.* (live feed). However, this practice is associated with several unavoidable problems. Live food organisms availability depends on the environmental factors. Hence they are not available throughout the year and collection from natural habitat is unpredictable, laborious as well as time consuming. On the other hand,artificial diets can be manufactured on a mass scale to ensure regular supply. Clariid fish larvae are reared upto fingerling size, either solely in a hatchery or only for 10-16 days period in a hatchery, followed

by nursery phase in the ponds for *C. gariepinus* (Polling *et al*., 1988, Britz and Hecht, 1988) and *C.magur* (Knud-Hansen *et al*., 1990). Chuapoehuk (1987) reported that minimum 30% of the dietary protein is required by the species for optimal growth and survival.

2.2. Major protein sources used in Aquafeed industry

In the recent years, intensification of fish culture has led to dependence on artificial feeds. Protein is the most important as well as an expensive component of aquafeed and also the key factor that affects the growth performance of fish and the cost of the feed (Lovell, 1989; Luo *et al.,* 2004). Dietary protein level is of fundamental importance, because it is going to significantly influence the survival, growth, and yield of the fish as well as the economics of farming industry by affecting the cost of the feed which is typically considered the main operational cost. Increased level in dietary protein has often been associated with comparatively higher growth rate in many fish species. However, beyond a certain level further growth is not supported, and may even decrease (Mohanty and Samantary, 1996; Kim and Lall, 2001; Debnath *et al.,* 2007). Traditionally fish meal (FM) is the main source of dietary protein used in aqua-feeds. Fish meal is often utilized in the aquafeed industry due to its well balanced essential fatty acids, indispensable amino acids, vitamins and minerals profile and generally, it enhances the palatability (Kaushik *et al*., 1995). Currently, about 20.2 % of the total global fish catch is used for the production of fish oil and fishmeal, and about 60.8% of total fishmeal is consumed by the aqua-feed industry (FAO, 2012). Fish oil and fishmeal produced from marine capture fisheries have decreased at annual average rates of 2.6 percent and 1.7 percent, respectively (FAO, 2012). In recent years, due to the decreasing availability, poor quality and increasing cost in the market has stimulated several studies on its partial or complete replacement of fish meal with alternative protein sources (Kaushik *et al.,* 1995; Fournier *et al.,* 2004). In most of the developing countries, this situation has made the aquaculture enterprises less profitable. So, there is a need for alternate sources of protein which is having a high protein content, well-balanced essential amino acids and high digestibility to cope up with these constraints. Therefore, utilization of alternate and inexpensive plant protein source will be beneficial in reducing the cost of feed and it will contribute to

food security (SOFIA, 2007). Presently, most of the commercial feed industry depends mainly on soybean meal as a fish meal replacer. Soy protein concentrates, wheat gluten and corn gluten meal had been found as a possible replacement of fishmeal in aquaculture diets. Partial replacement of fishmeal has been achieved by using plant protein concentrates as the sole protein source, in most cases with the supplementation of limiting amino acid or other nutrient. Use of alternate plant protein source to partially replace fish meal has been reported in Atlantic salmon, *Salmo salar* (Refstie *et al.,* 2001), cultured Atlantic halibut, *Hippoglossus hippoglossus* (Berge *et al.,* 1999), European sea bass, *Dicentrarchus labrax* (Tibaldi and Tulli, 1998), rainbow trout, *Oncorhynchus mykiss* (Alexis *et al.,* 1985), Japanese flounder, *Paralichthys olivaceus* (Kikuchi 1999), turbot, *Scophthalmus maximus (*Day and Plascencia-Gonzalez, 2000). Only in rainbow trout complete replacement of fishmeal with soy protein or wheat gluten has been achieved (Kaushik *et al.,* 1995; Rodehutscord *et al.,* 1995). Fishmeal was completely replaced in rainbow trout diets by using plant protein mixtures with a part of dietary protein supplied by other animal sources (Watanabe *et al.,* 1997). According to Kissil *et al.* (2000) 30% replacement offishmeal with soy protein, cause reduced growth in seabream while 30% of fishmeal protein was successfully replaced with corn gluten meal without affecting the growth (Robaina *et al.,*1997).

2.3. Potential alternative plant protein sources: Non-edible oil seed-based ingredients

The increasing demand for aquafeed and the high cost of conventional feed ingredients has led the researchers to search for alternative resources especially from plant material to meet the need for protein sources in the aquaculture sector. This search has been directed towards the ingredients that will not conflict with human food security, of which non-edible oils seed resources became most prominent and preferred choice. Among the potential plant ingredients that appear to be the best substitutes of fish meal are soya bean meal (SBM), lupin, peas, rapeseed meal and sunflower (Kaushik *et al.,* 1995), detoxified *Jatropha* kernel meal (DJKM) (Kumar *et al.,* 2010).

Suprayudi *et al.* (2015) indicated that the *Cyprinus carpio* fed with 50% de-fatted rubber seed meal gave a comparable growth performance and better feed intake corresponding to the soybean meal fed groups. Deng *et al.* (2015) reported that rubber seed meal could be included at level up to 30 % in the diets for tilapia without any adverse effects on the growth, antioxidant capacity and health status. According to Burel *et al.* (2000) extruded lupin found as a promising substitute for fish meal in the diets of trout and turbot, with an acceptable digestibility of its protein i.e., 96% in trout and 98% in turbot respectively. Davies *et al.* (1990) reported that practical inclusion limit of 15% rapeseed meal in tilapia feeds, as an alternate source of protein, did not produce any adverse effect. Different studies have shown that tilapia fed with Palm Kernel Cake which is pre-treated with commercial feed enzymes had better feed utilization and growth efficiency compared to the fish fed with similar levels of raw Palm Kernel Cake (PKC) (Ng *et al*., 2002). Up to 30%, enzyme-treated PKC can be incorporated into red tilapia diets without significant reduction in growth. The detoxified *Jatropha* kernel meal (DJKM) can replace 50% FM protein in common carp diets, without compromising the growth and health of fish (Kumar *et al.,* 2010). Heated *J. platyphylla* kernel meal (H-JPKM) is a good dietary protein source for Nile tilapia feed because growth performance and feed utilization of *Jatropha* group were similar to control group (FM fed group) and SBM group. Kumar *et al.* (2012) reported that five to six times increased fish body mass within 12 weeks. Detoxified *Jatropha* kernel meal can replace 50% FM protein in shrimp diets, without reducing growth and nutrient utilization parameters (Harter *et al.,* 2011). The results of these findings showed that non-edible seed cake or kernel meal after detoxification and with proper supplementation with deficient amino acid could be an excellent protein source in the diets of fish and other livestock animals.

2.4. *Jatropha curcas* (L)

Jatropha curcas, being a multipurpose tree, is of Central American and Mexican origin. Now it is also cultivated in many other Asian, Latin American and African countries. *Jatropha* genus belongs to the family Euphorbiaceae and has approximately 170 known species. *Jatropha curcas* is commonly known as purging nut or physic nut. This drought-resistant shrub, *J. curcas*, requires nearly 250 mm of annual rainfall and can survive in the stony and poor soil. It can be easily

propagated by seeding or cutting and it can reach a height upto 8 m very rapidly. This plant is mainly cultivated for the production of seeds. The average seed weight is 0.64 g and it contains 38–40% of oil indicating its potential as a renewable source of energy (Makkar *et al.,* 1998; Kumar and Sharma, 2008) that can be used in bio-diesel production. For optimum seed production, 900 to 1200 mm of annual precipitation is required. A yield of about 5 tons of seed/ha/year has been achieved (Makkar and beker, 1997) which is capable to produce 2 tons of biodiesel and 1 ton of seed meal rich in protein. The leaf extract of *J. curcas* can produce a potent cardiovascular action and the latex of the stem has antimicrobial property. The *Jatropha* fruit extract can terminate the pregnancy and has molluscicidal activity (Makkar and becker, 1998).

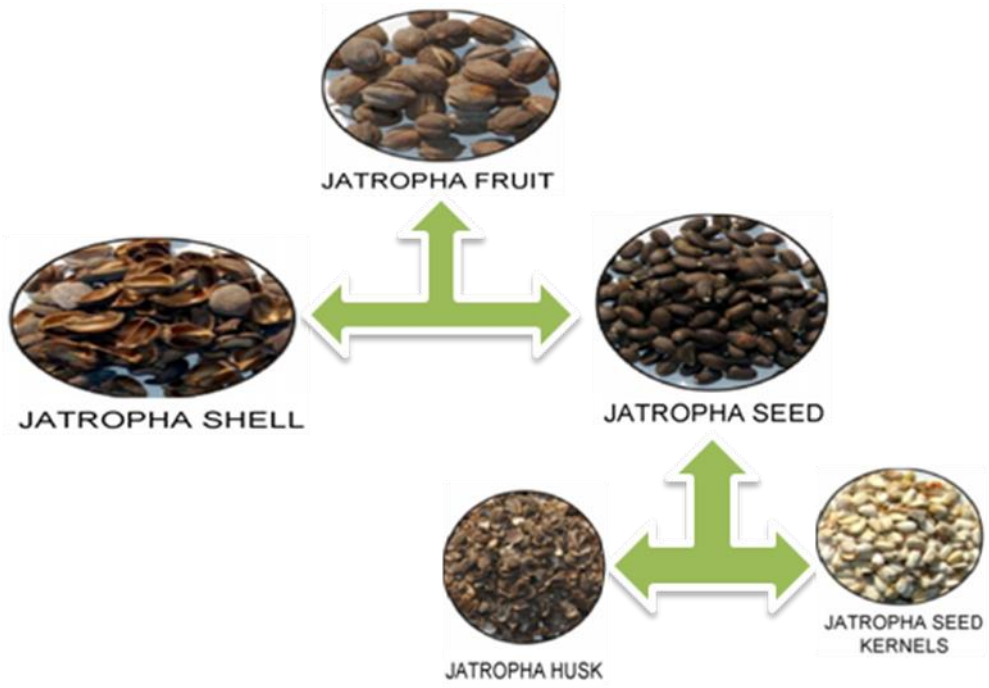

Plate 1. Process of obtaining *Jatropha* seed

2.5. *Jatropha* seed cake/meal–A potential alternative protein source

The *J. curcas* seeds are rich in oil (27.36%) and protein (32.88%), and it contains a significant amount of macrominerals (Na, K, Mg, Ca, P) and microminerals (Mn, Fe, Zn) (Abou-Arab and Abu-Salem, 2010). The *Jatropha* kernel meal which is obtained after oil extraction is an excellent source of nutrients and

contains 58–62% crude protein (Makkar *et al.,* 2008). Except lysine the levels of all essential amino acids are higher in *Jatropha* kernel meal than Soybean meal (Kumar *et al.,* 2010). The dry *Jatropha* fruit weighed approximately 2.1 g with a seed to husk ratio of 71:29 (w/w). The average seed weight is 0.64 g and the kernel constitutes a large proportion of the seed (61.3% by weight). *Jatropha* kernel is mainly composed of protein and lipid with a very little amount of moisture and ash. The shell of *Jatropha* seed mainly composed of fiber (84 to 89% natural detergent fibre). The low protein and high detergent content of the seed indicate its low nutritional value.

Table 1: Chemical composition of kernel and shell of *J. curcas*

Constituents(%DM)	Toxic variety		Nontoxic variety	
	Kernel	**Shell**	**Kernel**	**Shell**
Crude protein	22.2	4.3	27.2	4.4
Lipid	57.8	0.7	58.4	0.5
Ash	3.6	6.0	4.3	2.8
Natural detergent fibre	3.8	83.9	3.8	89.4
Acid detergent fibre	3.0	74.6	2.4	78.3
Acid detergent Lignin	0.2	45.1	0.0	45.6
Gross energy(MJ/kg)	30.7	19.3	31.1	19.5

(Adopted from: Makkar and Becker, 1999)

According to Makkar *et al.,* 1998 the crude protein content of defatted *Jatropha* kernel meal 56.4% in case of toxic variety and 63.8% in case of nontoxic variety which is more than that of commercial soybean meal. The essential amino acid composition of *Jatropha* kernel meal, except lysine, is higher than FAO reference protein.

Table 2: Essential amino acid composition of *J. curcas* seed meal as compared to castor bean, FAO reference protein (required for 2 to 5-year-old child) and soybean meal

Amino acids	Toxic variety	Non toxic variety	Castor bean	FAO reference protein	Soybean	Extracted meal (Moringna)
Lysine	4.28	3.40	3.86	5.80	6.08	1.48
Leucine	6.94	7.50	4.48	6.60	7.72	5.84
Isoleucine	4.53	4.85	6.27	2.80	4.62	3.49
Methionine	1.91	1.76	1.65		1.22	2.13
Cysteine	2.24	1.58	1.42	2.50	1.70	4.72
Phenylalanine	4.34	4.89	4.04		4.84	4.29
Tyrosine	2.99	3.78	2.65	6.30	3.39	1.41
Valine	519	5.30	5.53	3.50	4.59	3.63
Histidine	3.30	3.08	2.19	1.90	2.50	2.28
Threonine	3.96	3.59	3.35	3.40	3.76	2.28
Tryptophan	1.31			1.10	1.24	

(Adopted from: Makkar, and Becker, 1999)

2.6. Major anti-nutritional factors found in *J. curcas* by-products:

In *J. Curcas* seed meal a number of anti-nutritional factors are found. According to Makkar *et al.,* 2008*,* high levels of anti-nutritional factors such as

trypsin inhibitor, lectin and phytate are present in the seed meal of *J. curcas* and the major toxic component is phorbol esters (PES) (Makkar and Becker, 1997) which restrict their use in fish feed.Anti-nutrients such as protease inhibitors and lectins are heat labile and easy to inactivate by moist heating (Makkar and Becker, 2009).

2.7. Phytic acid

Phytate is an anti-nutritional factor found in most commonly used plant-derived ingredients of aqua-feed such as detoxified *Jatropha* kernel meal from the toxic genotype and *Jatropha* kernel meal from non-toxic genotype, soybean meal rice, barley, wheat, maize, sesame, groundnut and rapeseed (Makkar *et al.,* 1998; Makkar and Becker, 2009). The phytate or phytic acid content of *Jatropha* seed varies according to the variety (Reddy and Pierson, 1994). Consumption of *Jatropha* meal containing phytate can lead to decrease in the bioavailability of minerals (Oladele and Oshodi, 2007)*;* especially Zn and Ca. Phytates can also decrease the protein digestibility by forming complex substances and interacting with digestive enzymes such as pepsin and trypsin (Reddy and Pierson, 1994). The phytic acid content of *J. Curcas* seed has decreased significantly after soaking in distilled water. This reduction may be due to the leaching out of phytate ions into water under a concentration gradient, or changed permeability of *Jatropha* seed coat (Duhan *et al.,* 1989). The effect of phytate on the growth of the animals primarily depends on the amount present in the diet (Hossain and Jauncey, 1993). Phytate inclusion of 0.5% or 1% in purified diets of the agastric common carp (*Cyprinus carpio*) showed a significant reduction in feed efficiency and growth (Hossain and Jauncey, 1993). The specific growth rate of mrigal (*Cirrhinus mrigala*) and rohu (*Labeo rohita*) has significantly decreased when included more than 1% of the total diet (Usmani and Jafri, 2002). A diet of Channel catfish containing 2.2% phytate showed a significant reduction in feed efficiency, weight gain and Zn content in the vertebrae in compare to the fish fed with a diet containing 1.1% phytate (Satoh *et al.,* 1989). Feeding Atlantic salmon (*Salmo salar*) with high dietary phytate *i.e.,* 18 g phytate/kg, in the form of concentrated soybean meal, decrease the growth performance, protein and minerals (P, Zn, Mg and Ca) bioavailability as well as utilization (Storebakken *et al*., 1998). Phytate form chelate complex with divalent and trivalent mineral ions such as Mg^{2+} (Denstadli *et al.,* 2006), Ca^{2+}, Zn^{2+}

(Fredlund *et al.,* 2006), Fe^{3+}, Cu^{2+} andprevent their bioavailability for the growth of fish (Duffus and Duffus, 1991).

2.8. Tannins

Tannins are the substances contain multiple phenolic hydroxyl (OH) groups that lead to the formation of complexes mainly with proteins and with metal ions, amino acids, and polysaccharides, to a lesser extent.*J. curcas* kernel meal contains a negligible amount of tannins (0.02−0.04%). According to Makkar *et al.* (1998) the condensed tannins were absent in the *Jatropha* kernel meal, and in the bark, it was found to be very low in outer dark bark, 0.7% and inner green bark, tannins 3.1% (Makkar and Becker,2009).

2.9. Saponins

Saponins are triterpene glycoside or steroid compounds present in a variety of plants. In plants, saponins may serve in protecting the plant against fungi and microbes. However, often saponins taste bitter, so when it is present in very high concentrations, it will reduce the plant palatability in livestock (Sen *et al.,* 1998). The saponin content in *J. curcas* kernel meal from different regions ranged between 1.8 to 3.4% (as diosgenin equivalent). They are non-hemolyticin nature and the saponin content of non toxic and toxic varieties of *J. curcas* are almost same. These suggest that *J. curcas* saponins are the innocuous type compounds (Makkar *et al.,* 1997; 1998).

2.10. Lectins

Lectins are glycoprotein or carbohydrate-binding proteins and they are ubiquitous in nature. When the plant lectins are consumed by the animals, they will bind with glycosyl groups of the cells lining of the gastro intestinal tract membrane which leads to a series of systemic and harmful reactions. Such as (1) affecting gut epithelial cells, (2) interfering in digestion and nutrient absorption,(3) damaging the luminal membrane of the gut epithelium, (4) It will modulate the immunological status of the digestive tract (Francis *et al.,* 2001). Systemically, lectins disrupt protein, carbohydrate and lipid, metabolism, can cause atrophy or enlargement of internal organs, and alter the immunological and hormonal status. When it is

consumed at higher concentration, it may threaten the growth of the animals (Vasconcelos and Oliveira, 2004). Lectin content of the *J. curcas* kernel meal is 102 and 51 for toxic and nontoxic genotypes respectively (Makkar *et al.,* 2007).

2.11. Trypsin inhibitors

Trypsin inhibitors are found in many plant-based feed ingredients and they are responsible for the proteolytic enzyme inhibition in the intestinal tract, and therefore decrease the availability of amino acids for growth in cultured animals. Trypsin inhibitors are a kind of chemicals that can reduce the bioavailability of active trypsin which is very necessary for nutrition in animals including human beings. It will interfere with the physiological process such as digestion by influencing the normal function of the pancreatic proteolytic enzymes in cultured animals leading to severe growth depression.

The commonly cultured fish species have different ability to tolerate dietary trypsin inhibitor. Different inclusion levels of soybean meal, rapeseed meal, lupin seed meal and *Jatropha* seed meal, containing trypsin inhibitor were fed to the fishes. It is observed that Carp (Abel *et al*., 1984; Makkar and Becker, 1999),Tilapia (Shiau *et al.,* 1987, 1989; Wee and Shu, 1989.), rainbow trout (Rumsey *et al*.,1993; Dabrowski *et al*., 1989; and Krogdahl *et al*., 1994),seabream (Robaina *et al*., 1995) channel catfish (Wilson and Poe.,1985) and salmon (Higgs *et al*., 1982; Olli *et al.,* 1994) could maintain the growth rates compare to fish-meal based controls. 1.6 mg/g or higher of trypsin inhibitor in the diet retarded the growth of Nile tilapia, but the fish could tolerate and grew well at 0.6 mg Trypsin inhibitor/g of diet (Wee and Shu, 1989). Makkar and Becker (1999) reported that carp fed with diets containing *J. Curcas* seed meal of non-toxic provenance, having 24.8 mg TI/g and heat-treated *Jatropha* meal with 1.3- 8.3 mg Trypsin inhibitor/g, showed no differences in growth performance implies that the fish were able to tolerate the high dietary inclusion of Trypsin inhibitor. Rainbow trout have been found to be very sensitive to the trypsin inhibitors and there is a direct relation between the amount of trypsin inhibitor in the diet and protein and energy availability for trout (Krogdahl *et al.,* 1994). In Atlantic salmon trypsin production has been found in its peak level when the trypsin inhibitor level in the diet was 4.8 mg/g (Olli *et al.,* 1994). According to Wilson and Poe (1985) juvenile channel catfish showed best growth performance when fed with trypsin

inhibitor level of 2.2 mg/g of the diet. Krogdahl *et al.* (1994) reported that rainbow trout were able to partially compensate for trypsin inhibitor action by increasing the enzyme secretion and enhanced the protein absorption in the distal parts of the intestine. When the trout fed with trypsin inhibitor, ranging from 2.6 to 51.0 mg/g, observed a little effect on feed intake and growth performance when trypsin inhibitor levels were below 5 mg/g (Rumsey *et al*., 1995). It seems that most of the cultured fishes can compensate the presence of trypsin inhibitor action by increasing trypsin production, when it is present below 5 mg/g of diet. Norton (1991) reported that autoclaving for 15–30 min is a useful method to reduce the trypsin inhibitor level below the critical concentration. This heating process should be carefully done to minimise nutritional loss of the feed material, such as the loss of amino acids such as lysine and to prevent the reduction in protein degradability caused by excessive heat denaturation.

2.12. Phorbol esters and their toxicity

Phorbol esters are naturally occurring substances which are widely distributed in the plant species belong to the *Euphorbiaceae* family. Even though phorbol esters (phorbol-12-myristate 13- acetate) have been considered as the major toxic compounds in *Jatropha* (Makkar and Becker, 1997), but phorbol ester content depends on the soil quality and climatic conditions (Martinez-Herrera *et al*., 2006). The oil derived from the nontoxic (Mexican) varieties of *Jatropha* has been reported to contain an egligible amount of phorbol esters, around 0.27 mg/ml (Martinez-Herrera *et al.,* 2006). The biological effects of these toxic compounds are tumor promotion, adverse biochemical and cellular effects, alteration of cell morphology, it also induces platelet aggregation and serves as lymphocyte mitogens (Blumberg and Boutwell, 1980). The toxic compounds isolated from *Jatropha* oil had an irritant effect, caused diarrhea and mortality in the test animals (Gandhi *et al.,* 1995). *Jatropha* phorbol esters are biologically active and characterized by a 4ß-hydroxyl group. They form a group of at least 6 compounds, generally referred as *Jatropha* factors C1,C2,C3,C4,C5, C6 (Hua *et al*., 2015). Phorbol esters are cyclic diester form of dicarboxylic acids containing bicyclohexane (i.e., factors C1, C2, C4, C5) or cyclobutane (*i.e.,* factors C3 and C6) moieties. Among these, the most abundant derivative is factor C1 (Roach *et al*., 2012).

Phorbol esters are chemically unstable and they are prone to isomerisation, oxidation, photo degradation, and hydrolysis (Vogg *et al.,* 1999; Haas *et al*., 2002; Goel *et al.,* 2007; Roach *et al.,* 2012; Devappa *et al.,* 2013). *Jatropha* seeds containing the phorbol esters lead to severe irritation and extensive hemorrhages in the intestinal tract and congestions in other organs such as liver, kidney and lung, and focal necrosis in the heart and liver. Cooking of *Jatropha* seeds would destroy the heat-labile enzymes in it but not the phorbol esters and thus marginally reduced the toxic effect in rodents (Liberalino *et al.,* 1988). Hence it is assumed that most of the lesions caused due to *Jatropha* phorbol esters. Becker and Makkar (1998) reported that common carp (*Cyprinus carpio*) are highly sensitive to phorbol esters obtained from *Jatropha* seeds. The threshold level at which common carp showed negative effects was 15 µg PEs/g in feed and higher doses resulted in anorexia and reduction of growth rate. Kumar *et al*. (2011a) indicated that supplementation of partially purified phytate derived from *Jatropha* in the diet of fish @ 1.5% and 3% would affect the digestive physiology and growth performance in tilapia. In a recent study with rainbow trout (Kumar *et al.,* 2011b), the tolerance of the treated *Jatropha* kernel meal was reported. Treated *Jatropha* kernel meal (Makkar and Becker, 2010a) was used in a feeding trail to replace the fishmeal protein of the diet @50%and 62.5%. A 50% replacement showed no differences with respect to the control group (fishmeal protein set to 100%). The diets were supplemented with lysine and phytase to balance the amino acid composition difference between the two protein sources.

2.13. Detoxification methods used in the removal of anti-nutritional factors present in *Jatropha* by-products:

Among the anti-nutritional factors present in *Jatropha* kernel meal, lectins and trypsin inhibitors and are heat labile and can be destroyed by heat treatments. On the other hand, different studies showed that heat treatment is not alone effective in reducing phorbol ester content. Makkar and Becker (1997) reported 5% reduction in Phorbol Ester levels in the *Jatropha* kernel meal by heat treatment at 121°C for 30 minutes. Makkar and Becker (1998) reported that washing the heat treated kernel meal (121°C, 30 min, 66% moisture) (four times) with aqueous ethanol (80%) or aqueous methanol (92%) contains< 1% oil [1:5 w/v;

kernel meal: solvent] and *Jatropha* PEs reduced by 95%. Aregheore *et al.* (2003) observed that 95% reduction of PEs content in *Jatropha* kernel meal after heat treatment at 121°C for half an hour and followed by washing with aqueous methanol (92%) (four times). A reduction of 92% Phorbol Ester content was observed after alkali wash with 4% sodium hydroxide and sodium hypochlorite solution (10%) which is followed by heat treatment at 121°C for 30 min. According to Chivandi *et al.* (2004) double solvent extraction (i.e., hexane-ethanol system) along with wet extrusion at 126°C, 2 atmospheres for 10 min and re-extraction has done with hexane and heat (moist) at 121°C for 30 min reduced Phorbol ester level by 87.7%.

Martínez-Herrera *et al.* (2006) reported that use of aqueous ethanol (90%), which is followed by treatment with 0.07% $NaHCO_3$ and autoclaving at 121°C for 20 minutes could reduce PE content in *Jatropha* kernel meal by 98%, and 96% reduction was observed only by using 90% aqueous ethanol. Rakshit *et al.* (2008) stated that treated *Jatropha* kernel meal showed 90% and 88% reduction in Phorbol ester content. Gaur (2009) applied the principle called solid-liquid extraction to treat ground *Jatropha* seed kernels which showed a reduction in phorbol ester content by 99.6%. Makkar and Becker (2010a), reported a method which involves extraction and inactivation of phorbol esters in *Jatropha* kernel meal using 70 to 90% aqueous methanol having 0.05 -0.2 M sodium hydroxide at 50–70°C for 1 hour followed by organic solvent washing. The concentration of phorbol ester in the resultant material was <3 mg/kg. Li *et al*. (2015) observed a reduction in the concentration of phorbol ester present in *Jatropha* kernel meal by 85.5%, using a steam treatment which is followed by extraction with ethyl alcohol at 55°C for 2 hours. Nokkaew and Punsuvon (2013) reported the use of aqueous ethanol (concentration not given) to remove phorbol ester from the hexane-de-oiled kernel meal. It was observed a reduction in the PEs by 96.6% in a two-stage extraction at 1:3 (w/v) de-oiled meal to aqueous ethanol at 50°C for 30 minutes. Solid state fermentation is found to be one of the methods for detoxifying plant products which contain tannin and phytate (Madeira *et al.,* 2011), soybean bran (Wolski *et al.,* 2009) and *Jatropha* seed cake (Belewu and Sam, 2010; Joshi *et al.,* 2011). The fermentation study using *Pseudomonas aeruginosa (*PseA) in *Jatropha* seed cake showed a positive result (Joshi *et al.,* 2011).

2.14. Effect of detoxified Jatropha byproducts on animal response and Performance

Detoxified *Jatropha* kernel meal (DJKM) is a good protein source for carp (*Cyprinus carpio*) (Kumar *et al.,* 2008) and rainbow trout (*Oncorhynchus mykiss*) diets (Kumar *et al.,* 2011b).Feeding trials on common carp and rats with *Jatropha* meal containing phorbol esters (PEs) reported to cause marked reduction in feed intake, diarrhea and growth depression in the test animal (Rakshit *et al.,* 2008). Growth performance of carp fed with detoxified *J. curcas* kernel meal, using heat treatment for 60 min (50% FM protein replacement by DJKM) was better than of those fed with soy bean meal based diets and are similar to those fed with fish meal based diets (Kumar *et al*., 2010). The inclusion of DJKM in common carp could reduce cholesterol level in plasma and muscle as compared to control (FM-diet) (Kaushik *et al.,* 1995; Yamamoto *et al.,* 2007). According to Kumar *et al.* (2011b) rainbow trout were efficient in digesting protein, lipid and energy from the DJKM meal at 50% replacement level compare to the fish fed at higher inclusion level (62.5% replacement of FM). Crude protein digestibility of DJKM diets was high (above 84%) in rainbow trout, suggesting DJKM to be an excellent protein source for this species (Kumar *et al.,* 2011b). Harter *et al.* (2011) reported that 50% fish meal protein in shrimp diets could be replaced by detoxified *Jatropha* kernel meal, without sacrificing growth and nutrient utilization parameters. Overall, the nutrient utilization and growth performance of white leg shrimp (*i.e., L. vannamei)* to detoxified *Jatropha* Kernel meal fed groups were better than fish meal-fed groups which indicate that shrimp can efficiently use detoxified *Jatropha* kernel meal.

Heat-treated *Jatropha phytophylla* kernel meal (H-JPKM) was tested as a protein supplement in the feed of tilapia and compared to the soybean meal and fish meal. Kumar *et al.* (2012) concluded that Nile tilapia fingerlings fed with control diet containing fishmeal, and the test diets where 62.5% of FM protein was replaced by *Jatropha* and Soybean meal showed no significant difference in feed conversion ratio, protein efficiency ratio and in the growth performance which indicates that *J. phytophylla* kernel meal can be used as a protein source in fish feed.

Saha and Ghosh (2013) demonstrated the suitability of raw and fermented deoiled *J. Curcas* seed meal (DJSM) as alternative protein source ingredient for *Labeo rohita* (rohu). Their findings revealed that feeding rohu with raw DJSM resulted in a progressive decline in specific growth rate, weight gain (%), protein efficiency ratio, apparent protein digestibility and digestive enzyme activities. However, the fish fed with fermented DJSM exhibited similar performance with the control in terms of growth and nutrient utilization parameters, thus, the authors proposed that an inclusion level up to 30% of fermented DJSM replacing 15% FM can be incorporated in the practical diet for rohu fingerlings without compromising growth, feed utilization efficiency and whole body composition (Saha and Ghosh, 2013). Shamna *et al.* (2015) evaluated the nutritional potential of fermented *Jatropha* protein concentrate (FJPC) compared to soybean protein concentrate. The authors stated that FJPC detoxified by solid state fermentation could be included up to 20% in the diets for *L. rohita* fingerlings without compromising growth and nutrient utilization, but reduction in percent weight gain, protein efficiency ratio and lower survival rate were recorded in non-fermented JPC fed groups, and the reason was described to the high level of phorbol ester present in JPC (1.4 mg g-1). Hence it was concluded that solid state fermentation using *Aspergillus niger* is an effective method for the removal of PEs for efficient protein utilization.

3. MATERIALS AND METHODS

3.1. Experimental Site

The experiment was conducted at the Freshwater Fish Farm of ICAR-CIFE, Balabhadrapuram, Kakinada Centre, Kakinada, East Godavari District, Andhra Pradesh.

3.2. Experimental Fish

Fingerling of *Clarias magur was* used as an experimental fish. The fish were procured from the freshwater fish farm located in Balabhadrapuram. The fish were disinfected with $KMnO_4$ solution. Then the fish were acclimatized for a period of 7 days and fed with supplementary feed.

3.3. Experimental Design

A completely randomized design (CRD) was followed for the experiment. The experiment was designed with one control and three treatments, each was triplicated.

3.4. Experimental Setup

Twelve FRP tanks, each with 300 L capacity, were used as experimental tanks. Prior to stocking, the tanks were dewatered and treated with bleaching powder for disinfection. Then the tanks were washed in freshwater and sun dried.

Fresh water obtained from the bore-well of ICAR-CIFE, Balabhadrapuram farm was used to rear the fish. A total of 20 number of fish were stocked in each tank. The initial length and weight of stocked fish were 8±0.5 cm and 5±0.5 g, respectively.

Plate 2: Experimental setup

3.5. Feeding

Fish were fed with formulated experimental diets during the experimental period. Feed was given twice a day (@ 5% body weight), during morning and evening at 07.00 hrs and 17.00 hrs respectively.

3.6. Formulation of feed

Four iso-nitrogenous experimental diets were prepared by using detoxified *Jatropha curcas* kernel meal to replace soybean meal on a dry weight basis. Inclusion level of Jatropha were 0%, 10%, 20% and 30% in diets. The diet containing 0% *J. curcas* kernel meal is used as a control diet. For preparing the experimental diets, all other ingredients were mixed. The mixed ingredients were made into a dough by adding water and cooked for 45 minutes. Vitamin-mineral premix, oil, BHT and choline chloride were added later. Then the dough was passed through a hand pelletizer machine. The pellets were sun-dried until it reaches a constant weight. Proximate composition of *J. curcas* kernel meal and anti-nutritional factors are shown in Table 3 & 4.

Table 3: Proximate composition of *J. curcas* kernel meal (%)

Parameters	Values (%)
Moisture	6.19 ± 0.98
Crude protein	48.5 ± 5.23
Ether extract	2.14 ± 0.45
Crude ash	10.43 ± 1.11
Crude fibre	9.92 ± 1.03
NFE	29.01 ± 2.26

Table 4: Anti-nutritional factors (ANFs) present in *Jatropha* kernel meal

Test parameters	Test result (% DM)
Tannin	6.1 ± 0.001
Total phenol	6.1 ± 0.02
Phytate	0.1 ± 0.001
Trypsin inhibitor (TI)	0.072 ± 0.001
Phorbol ester (PE)	ND

DM= Dry matter, ND= Not Detected

Plate 3: Mixing of feed ingredients

Plate 4: Preparation of experimental feed

Plate 5: Experimental feeds

Table 5:Composition of the experimental diets (%)

Ingredients	control	T1	T2	T3
fish meal	**5**	**5**	**5**	**5**
SBM	**30**	**20**	**10**	**0**
Jatropha	**0**	**10**	**20**	**30**
GNOC	**30**	**30**	**30**	**30**
MOC	**10**	**18**	**18**	**18**
Ricebran	**10**	**3**	**3**	**3**
Wheat flour	**5**	**2**	**2**	**2**
corn flour	**5**	**2**	**2**	**2**
VITAMIN-min	**2**	**2**	**2**	**2**
Choline chloride	**0.3**	**0.3**	**0.3**	**0.3**
CMC	**1.675**	**1.675**	**1.675**	**1.675**
BHT	**0.025**	**0.025**	**0.025**	**0.025**
OIL	**6**	**6**	**6**	**6**

C=Control, T1= 10% inclusion of Jatropha, T2= 20% inclusion of Jatropha, T3= 30% inclusion of Jatropha

Table 6: Proximate composition of the experimental diets (%)

Components	**C**	**T1**	**T2**	**T3**
Moisture	8.52 ± 0.01	8.07 ± 0.12	8.45 ± 0.11	8.20± 0.31
Crude protein	35.23 ± 0.21	35.21 ± 0.15	35.13 ± 0.30	35.63 ± 0.19
Crude lipid	7.27 ± 0.08	7.15 ± 0.24	6.91 ± 0.47	7.22 ± 0.32
Ash content	6.38 ± 0.48	6.28 ± 0.21	5.97 ± 0.19	5.88 ± 0.22
Crude fibre	3.77 ± 0.19	3.56 ± 0.23	3.78 ± 0.37	3.83 ± 0.19
NFE	38.81 ± 0.25	39.70 ± 0.18	39.74 ± 0.81	39.25 ± 0.92
Digestible energy(kcal/100g)	361.66 ± 2.55	364.06 ± 2.86	361.69± 1.88	364.55 ± 1.45

C=Control, T1= 10% inclusion of Jatropha, T2= 20% inclusion of Jatropha, T3= 30% inclusion of Jatropha

3.7. Chemicals and Glasswares

The glasswares, chemicals and kits used throughout the experiment were supplied by the Genetix Biotech Asia Pvt. Ltd and Genex Life Sciences Pvt. Ltd.

3.8. Water Quality Analysis

Water quality parameters namely dissolved oxygen, pH, hardness, total alkalinity, ammonia, and nitrate were monitored once in 10 days during the experimental period by using test kits (Advance Pharma Co., Ltd, India; Nice Chemicals Pvt. Ltd, India; U International Company, India) (APHA, 1998).

3.9. Growth analysis

Growth sampling was done at fortnight interval by collecting the fish using hand net. Each time, 100% of the stocks were sampled from each tank to record the length and weight of the individual fish. The total length of fish from tip of the snout to end of the caudal fin was measured using a meter scale with an accuracy of one millimetre. The total weight of fish was measured using a weighing balance with an accuracy of 0.1 g.

3.9.1. Percentage Weight Gain

Percentage weight gain has been calculated using the following formula:

$$\text{Percentage weight gain (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

3.9.2. Specific Growth Rate (SGR)

The specific growth rate has been calculated by the following formula:

$$\text{SGR (\%/ day)} = \frac{\text{(ln Final weight} - \text{ln Initial weight)}}{\text{Number of days}} \times 100$$

3.9.3. Feed Conversion Ratio (FCR)

Feed conversion ratio was estimated by the following formula-

$$\text{Feed Conversion Ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

3.9.4. Feed Efficiency Ratio (FER)

The feed efficiency ratio was calculated by the following formula –

$$\text{Feed Efficiency Ratio} = \frac{\text{Body weight gain (wet weight)}}{\text{Feed given (dry weight)}}$$

3.9.5. Protein Efficiency Ratio (PER)

The protein efficiency ratio was calculated by the following formula

$$\text{Protein Efficiency Ratio} = \frac{\text{Body weight gain (wet weight)}}{\text{Total protein fed}}$$

3.9.6. Survival Rate (%)

At the end of the experiment all the culture tanks were dewatered and the number of experimental fish in each tank was counted and the survival rate (%) was calculated by the following formula:

$$\text{Survival (\%)} = \frac{\text{Total number of fish harvested}}{\text{Total number stocked}} \times 100$$

3.9.7. Net protein utilization

NPU can be calculated by using the following formula:

$$\text{Net Protein Utilization} = \frac{\text{Nitrogen retained}}{\text{Nitrogen intake}} \times 100$$

3.10. Tissue sampling and tissue homogenate preparation

At the end of the experiment five fish from each replicate (15 fish per treatment) were sacrificed for tissue collection for biochemical and physiological analysis. For digestive enzyme analysis, the samples were taken 1 h after the last feeding to guarantee maximum activity of digestive enzymes. The liver, intestine and muscle of the fish were dissected out aseptically and were homogenized with 0.25 M chilled sucrose solution on wet basis (pH 7, 1:10 w/v) using tissue homogenizer (Remi, India). The tube was continuously kept in ice to avoid heating. Then homogenate was centrifuged at 8000 rpm for 10 min at 4°C in a refrigerated centrifuge (Eppendorf, Germany). After centrifugation, the top lipid layer was removed and the supernatant solution was divided as aliquots in 2 ml centrifuge tubes. The samples were stored at −20°C until further analysis.

Plate 6: Weight measurement during sampling

Plate 7: Length measurement during sampling

3.11. Oxidative Stress Parameters

3.11.1. Superoxide Dismutase Assay

The Superoxide Dismutase (SOD) activity was estimated by the method of Mishra and Fridovich (1972) with slight modifications. Liver tissue was used for the analysis of SOD activity. The assay is based on the oxidation of epinephrine adrenochrome transition with the help of enzyme. The reaction mixture having 50 µl of the sample,1.5 ml of phosphate buffer and 0.5 ml epinephrine. The solution was mixed well and the change in OD at 480 nm for 2 min was observed in UV spectrophotometer. One unit of SOD activity was the amount of protein required to give 50% inhibition of epinephrine auto oxidation.

3.12.2. Catalase Assay

Catalase activity (CAT) was estimated following the method of Takahara *et al.* (1960). Liver tissue was used for the analysis of CAT activity. 2.45

ml of phosphate buffer (50 mM, pH 7.0), and 50 µl of the tissue homogenate were mixed together and the reaction was initiated by the addition of 1.0 ml H_2O_2 solution. The decrease in the absorbance value was measured at 240 nm at 30 sec intervals for 2 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The CAT activity was expressed as nmoles of H_2O_2 decomposed/ min/ mg protein.

3.13. Metabolic response

3.13.1. Aspartate Aminotransferase Activity (AST)

The AST activity was assayed in liver tissue homogenate as described by Wootton (1964). The substrate comprised of 0.2 M D, L- aspartic acid and 2 mM α-keto glutarate in 0.05 M phosphate buffer (pH 7.4). 0.5 ml of substrate was added In the experimental tubes and control tubes. The reaction was initiated by adding 0.1 ml of tissue homogenate. The mixture was incubated at 37°C for 60 minutes. 0.5 ml of 1 mM 2, 4-dinitrophenylhydrazine (DNPH) was added to stopthe reaction. The enzyme source was added in the control after DNPH solution. The tubes were held at room temperature for 20 minutes with occasional shaking. Then, 5 ml of 0.4 M NaOH solution was added, the contents were thoroughly mixed. After 10 minutes, the OD was recorded at 540 nm against blank. The activity was expressed as nano moles of oxaloacetate released min^{-1} mg^{-1} protein at 37°C.

3.13.2. Alanine Transaminase Activity (ALT)

The ALT activity was assayed in liver tissue homogenate as described by Wootton (1964). The procedure adopted for ALT activity was same as for AST activity except the substrate comprised of 0.2 M D, L- alanine instead of aspartic acid. The activity was expressed as nano moles of sodium pyruvate released min^{-1} mg^{-1} protein at 37°C.

3.13.3. Alkaline Phosphatase assay (ALP)

The ALP activity of liver tissue was determined by the method of Garen and Levinthal (1960). The assay mixture comprised of 0.2 ml bicarbonate buffer (0.2 M), 0.1 ml of 0.1 M $MgCl_2$, 0.1ml tissue homogenate, 0.5 ml of distilled

water and 0.1 ml of freshly prepared 0.1M para-nitrophenyl phosphate. The mixture was kept for incubation in a water bath at 37°C for 15 min and the reaction was stopped by 1.0 ml of 0.1 N NaOH and OD was taken at 410 nm.

3.14. Digestive Enzymes

3.14.1. Amylase

The amylase activity of the intestine sample was determined using the DNS method (Bernfeld, 1955). Due to the action of glucoamylase and α-amylase on carbohydrate, the reducing sugars produced, was estimated using 3, 5-Dinitrosalicylic acid (DNS) method. The reaction mixture consists of 1% (w/v) starch solution, 0.1 M phosphate buffer (pH 7.0) and the tissue homogenate. The mixture was kept for incubation at 37°C for 30 min. After incubation DNS was added and kept for 5 min in boiling water bath. After getting cool, the reaction mixture was diluted with distilled water and absorbance was measured at 540 nm in a UV–VIS spectrophotometer (Biochrom, UK). Maltose was used as a standard. One unit of amylase activity was defined as the number of moles of maltose released from starch per minute per milligram of protein at 37°C.

3.14.2. Protease

The protease activity of the intestine sample was determined by the casein digestion method (Drapeau, 1976). The reaction mixture consisted of 1% casein in 0.05 M trisphosphate buffer (pH 7.8) and tissue homogenate. Then the mixture was incubated at 37°C for 10 minutes. Ten minutes later, the reaction was stopped by adding 10% TCA and the whole content was filtered using filter paper. The reagent blank was made by adding tissue homogenate just before stopping the reaction and without incubation. The absorbance was measured at 240 nm in a UV–VIS spectrophotometer (Biochrom, UK). The protease activity was determined from the tyrosine standard curve and expressed as micromole of tyrosine released min^{-1} mg^{-1} protein at 37°C.

3.14.3. Lipase

The lipase activity of intestine tissue was determined based on the method described by Cherry and Crandell (1932). The reaction mixture consists of distilled water, tissue homogenate, 0.1 M phosphate buffer (pH 7.0) and olive oil emulsion. After shaking the mixture was incubated at 27°C for 24 h. Then, 95% alcohol and two drops of phenolphthalein indicator were added and titrated against 0.05 N NaOH until the appearance of permanent pink colour. Control was taken using enzyme source that was inactivated prior to the addition of buffer and olive oil emulsion. One unit of lipase activity was considered as the number of micromoles of fatty acids released per minute per milligram of protein.

3.15. Proximate analysis of the experimental diets and fish carcass tissues

Proximate analysis of the experimental diets and carcass tissue were done following standard methods of AOAC (1995).

3.15.1. Moisture

The moisture content of the diets and fish carcass tissue was estimated by taking a known amount of sample in the petri dish and drying it in a hot air oven at 100- 105^{o}C till a constant weight was achieved. The difference in weight of the sample gave the moisture content, which was calculated using the following formula

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample} - \text{Dried weight of sample} \times 100}{\text{Wet weight of sample}}$$

3.15.2. Crude protein (CP)

The nitrogen content of the feed and tissue samples was estimated quantitatively by Kjeltec semi-automated method (2200 Kjeltec Auto Distillation, Foss Tecator, Sweden). The crude protein percentage was obtained by multiplying the nitrogen percentage by a factor of 6.25.

Crude protein (%) = N_2 (%) × 6.25

3.15.3. Ether Extract (EE)

Ether extract of the samples was estimated by Soxhlet apparatus using petroleum ether (Boiling point 55 ± 5^{o}C) as the solvent. The calculation was made as follows.

$$\text{Ether Extract (\%)} = \frac{\text{Weight of ether extract}}{\text{Weight of the sample}} \times 100$$

3.15.4. Ash

Ash content of the diets and fish carcass tissue was determined by using a known weight of the dried sample in a silica crucible and keeping it in a muffle furnace at 600^{o}C for 6 hrs. The calculation was done as follows:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.16.5. Total Carbohydrate (TC)

The total carbohydrate (TC) of the samples was calculated by subtracting the percentage of other nutrients from 100 (Hasting, 1969).

TC (%) =100 – Crude protein (%) – Ether extract (%) – Ash (%)

3.15.6. Digestible Energy (DE)

Digestible energy of the diets and fish carcass tissue was calculated as per standard physiological values (Halver, 1976) according to the formula.

Digestible energy (Kcal/100g) = Protein% x 4 + Lipid% x 9 + Carbohydrate% x 4

3.16. Analysis of Anti-nutritional factors

3.16.1. Phorbol ester

Phorbol esters was analysed by using the modified method of Saetae and Suntornsuk (2011). 5 g of sample was taken in 20 ml methanol and mixed it using an orbital shaker at 200 rpm and filtered using a whatman No. 1 filter paper and the filtrate was collected and then concentrated in a rotary evaporator at 65 °C. In HPLC (Waters, Massachusetts, USA the sample was quantified by using UV detector at 280 nm. The PE content was expressed as g/kg.

3.16.2. Phytic acid

Phytic acid estimation was done by using Vaintraub and Lapteva (1998) method. 1 g of sample was taken in Erlenmeyer flask. 50 ml of 3% trichloroacetic acid was added to it and after proper mixing it was centrifuged at 3000 g for 10 minutes. 8 ml of $FeCl_3$ solution was rapidly added to a 20 ml aliquot. This mixture was kept for boiling in a water bath for 45 min. The solution was again centrifuged at 3000 g for 10–15 min. After washing with 3% TCA, the precipitate was dispersed in few millilitre of distilled water and 6 ml of 1.5 N NaOH. The solution was made upto 120 ml and filtered through a Whatman No. 2 filter paper and made the precipitate dissolve in 400 ml volumetric flask containing 80 ml hot 3.2 N HNO_3. After cooling, the volume was made to 200 ml with distilled water. From this, 10 mL aliquot was made to 200 ml using 40 ml 1.5 M KSCN and distilled water. The reading was measured at 480 nm in UV-visible spectrophotometer (Shimadzu, UV1800, Kyoto, Japan) and a blank with each sample was run.

3.16.3. Total phenol

The total phenolic content was determined by using Folin-Ciocalteu reagent following a s modified method of Ainsworth and Gillespie (2007). For reference standard for plotting calibration curve Gallic acid was used. A volume of 0.5 ml sample was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The mixture was kept for incubation at room temperature with intermittent shaking for colour development. The absorbance was then measured at

765 nm using double beam UV-VIS spectrophotometer (UV Analyst-CT 8200). The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract.

3.16.4. Trypsin inhibitor

The method used for trypsin inhibitor estimation is a modified method of Makkar *et al.* (2007). 1 g of finely ground sample was taken and mixed with 50 ml of 0.01 M NaOH and stirred for 3 hours in room temperature at a pH range of 8.4-10. Aliquots (0, 0.6, 1.4 and 1.8 ml) of suspensions were taken and made upto 2.0 ml with distilled water. 2 ml of trypsin solution (Sigma, St. Louis, USA) was added and the tube were placed at 37 °C for 10 minutes. Termination of the reaction was done by the addition of 1ml, 30% acetic acid. The contents were centrifuged and absorbance was measured at 410 nm in UV-visible spectrophotometer (Shimadzu, UV1800, Kyoto, Japan) against the reagent blank. The activity was expressed in trypsin inhibitor units (TIU).

3.16.5. Tannin

Total tannin was quantified by using spectrophotometric method described by Makkar *et al.* (2007). The reagent, Folin-Ciocalteu was used and the results were expressed as tannic acid equivalents.

3.17. Statistical Analysis

Statistical analysis of different growth and physiological parameters were analysed by one-way analysis of variance (ANOVA) using SPSS version 20.0. Duncan's multiple range test was used for post hoc comparison of mean ($P<0.05$) between different treatment groups. All the data presented in the text, tables, and figures expressed as mean ± standard error (SE) and for the test statistical significance was set as $P<0.05$.

4. RESULTS

4.1. Water quality parameters

The physico-chemical parameters such as temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and hardness were recorded for each experimental tank and the ranges of all the parameters are presented in the Table 7.

4.1.1. Temperature (°C)

The temperature of the different experimental tanks ranged between 23 -27°C throughout the 60 days trial.

4.1.2. pH

The pH value of the water of different tanks was observed in the range of 7-8.4 during the trial period.

4.1.3. Dissolved oxygen

The dissolved oxygen content of all the experimental tanks was recorded within the range of 5.2-7.8 ppm during the 60 days trial.

4.1.4. Total alkalinity

The total alkalinity value was recorded within the range of 153 -165 ppm in all the experimental tanks throughout the trial period.

4.1.5. Total Hardness

Total hardness value was found to be within the range of 150 -161 ppm during 60 days trial period.

4.1.6. Ammonia-N (ppm)

Ammonia-N value was recorded within the range of 0.02- 0.07 ppm in all the experimental tanks throughout the trial period.

4.1.7. Nitrite-N (ppm)

Nitrite-N value was found to be within the range of 0.001- 0.005 ppm during 60 days trial period.

4.1.8. Nitrate-N (ppm)

Nitrate-N value was recorded within the range of 0.02- 0.05 ppm in all the experimental tanks throughout the trial period.

Table 7: Water quality parameters (Mean±SE) observed during 60 days experimental period

Parameters	C	T1	T2	T3
Temperature (°C)	24.8 ± 0.31	25.3 ± 0.28	24.8 ± 0.24	26.04± 0.22
pH	7.8 ± 0.01	7.8 ± 0.01	7.7 ± 0.005	7.8 ± 0.1
Dissolved oxygen (ppm)	6.5 ± 0.06	6.0 ± 0.08	6.6 ± 0.15	6.8 ± 0.06
Total alkalinity (ppm)	153.68 ± 0.45	154.51 ± 0.26	155.14 ± 0.59	155.89 ± 0.58
Total hardness (ppm)	155.49 ± 0.24	159.18 ± 0.24	158.07 ± 0.97	158.30 ± 0.35
Ammonia (ppm)	0.05 ± 0.01	0.02 ± 0.005	0.07 ±.001	0.02 ± 0.02
Nitrite (ppm)	0.005 ± 0.001	0.003 ± 0.01	0.001 ± 0.07	0.001 ± 0.02
Nitrate (ppm)	0.03 ± 0.02	0.05 ± 0.01	0.02 ± 0.003	0.05 ± 0.05

C=Control, T1= 10% inclusion of Jatropha, T2= 20% inclusion of Jatropha, T3= 30% inclusion of Jatropha. Data expressed as Mean ± SE, n = 3.

4.2. Growth parameters

The growth parameters of *Clarias magur* in different treatments had shown a varying trend throughout 60 days culture period. Initial average weight, final average weight and SGR of the fish recorded during the experiment are given in Table 8. Higher final average weight and specific growth rate were observed in T1 compared to fishes reared in other treatment groups.

4.2.1. Average body weight

The highest average body weight was observed in T1 (105.00 ± 1.32 g) and the lowest final average body weight was observed in T3 (93.16 ± 1.30 g) (Fig.1). There was a significant difference ($P<0.05$) in the weight gain of fishes reared in different treatments.

4.2.2. Percentage growth rate

The percentage growth rate of the different treatment groups is given in Table 8 and Fig. 2. At the end of 60 days, the lowest value was observed in T3 (44.82 ± 0.59) and the highest percentage growth rate was observed in T1 group (51.78 ± 0.69), which was significantly higher than T2 and T3.

4.2.3. Percentage weight gain

The percentage weight gain of the different treatment groups observed in the present study is given in Table 8 and Fig. 3. The significantly lowest value was observed in T3 (81.28 ± 1.96) and the highest percentage growth rate was observed in T1 group (107.51 ± 3.02).

4.2.4. Specific growth rate (SGR)

The specific growth rate observed during 60 days experimental period is presented in Table 8 and Fig. 4. There was a significant difference ($P<0.05$) in SGR of the fishes reared under different experimental treatment. The highest specific growth rate was observed in T1 (1.21 ± 0.02) and lowest in T3 (0.99 ±0.0).

4.2.5. Feed conversion efficiency (FCE)

The FCE obtained from 60 days culture of *C. magur* is presented in Table 8 and Fig. 5. The highest FER value was showed by T1 (0.41 ± 0.00) followed by T2 (0.37 ± 0.01). The fishes reared in T3 showed lowest FER value (0.35 ± 0.01) which was significantly lower than fishes reared in control and T1 group.

4.2.6. Feed conversion ratio (FCR)

The FCR observed in 60 days culture period is shown in Table 8 and Fig. 6 The lowest FCR value was observed in T1 (2.40 ± 0.05) followed by T2 (2.67 ± 0.04). The highest FCR value (2.85 ± 0.06) was found in T3 which was significantly different ($P<0.05$) from control and T1 groups.

4.2.7. Protein efficiency ratio (PER)

The PER values of *C. magur* is represented in Table 8 and Fig. 7. The highest PER value was showed by T1 group (0.43 ± 0.02) reared fishes. Fishes reared in T2 and T3 showed lower PER value which was significantly lower than the other group fishes.

4.2.8. Net protein utilisation (NPU)

The NPU values of *C. magur* is represented in Table 8 and Fig. 8. The highest NPU value was showed by T1 group (51.52 ± 0.24) reared fishes. Fishes reared in T2 and T3 showed lower NPU value which was significantly lower than the other group fishes.

4.2.9. Survival rate (%)

The Survival rate of the different treatment groups is given in Table 8 and Fig. 9. At the end of 60 days, survival rate (in percentage) was calculated. The highest survival rate was observed in control and T1 group (98.23 ± 1.02) followed by T2 (89.66 ± 1.37), which was significantly higher than other treatments.

Table 8: Growth Parameters of *Clarias magur* reared under different experimental diets

Growth parameters	C	T1	T2	T3
Initial weight (g)	50.91 ± 0.46	50.60 ± 0.21	50.87 ± 0.14	51.39 ± 0.23
Final weight (g)	108.00^{a} ± 2.29	105.00^{a} ± 1.32	96.00^{b} ± 1.04	93.16^{b} ± 1.30
Growth rate (%)	52.80^{a} ± 1.37	51.78^{a} ± 0.69	47.00^{b}± 0.44	44.82^{b}± 0.59
Weight Gain (%)	112.23^{a} ± 6.11	107.51^{a} ± 3.02	88.70^{b} ± 1.58	81.28^{b} ± 1.96
Specific Growth Rate(% day^{-1})	1.25^{a} ± 0.04	1.21^{a} ± 0.02	1.05^{b}± 0.01	0.99^{b}± 0.01
Feed Conversion Efficiency	0.42^{a} ± 0.01	0.41^{a} ± 0.02	0.37^{b} ± 0.02	0.35^{b} ± 0.01
Feed Conversion Ratio	2.33^{b} ± 0.09	2.40^{b} ± 0.05	2.67^{a} ± 0.04	2.85^{a} ± 0.06
Protein Efficiency Ratio	0.46^{a} ± 0.01	0.43^{a} ± 0.02	0.31^{b} ± 0.01	0.22^{b} ± 0.01
Net Protein Utilization	59.75^{a} ± 0.18	51.52^{a} ± 0.24	40.04^{b} ± 0.88	34.12^{b} ± 0.19
Survival rate (%)	100^{a} ± 0.01	98.23^{a} ± 1.02	89.66^{b} ± 1.37	89.04^{b} ± 0.79

C=Control, T1= 10% inclusion of Jatropha, T2= 20% inclusion of Jatropha, T3= 30% inclusion of Jatropha

Values in the same rows with different superscripts differ significantly ($P<0.05$) for each parameter. Data expressed as Mean ± SE, n = 3.

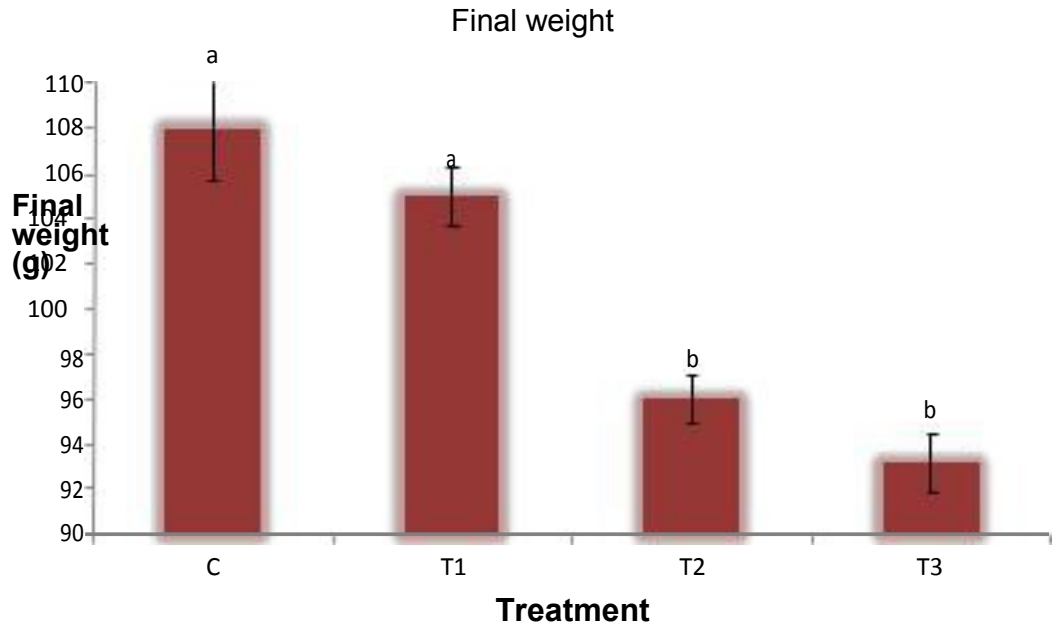

Fig. 1. Final body weight (g) of *C. magur* reared under different treatments

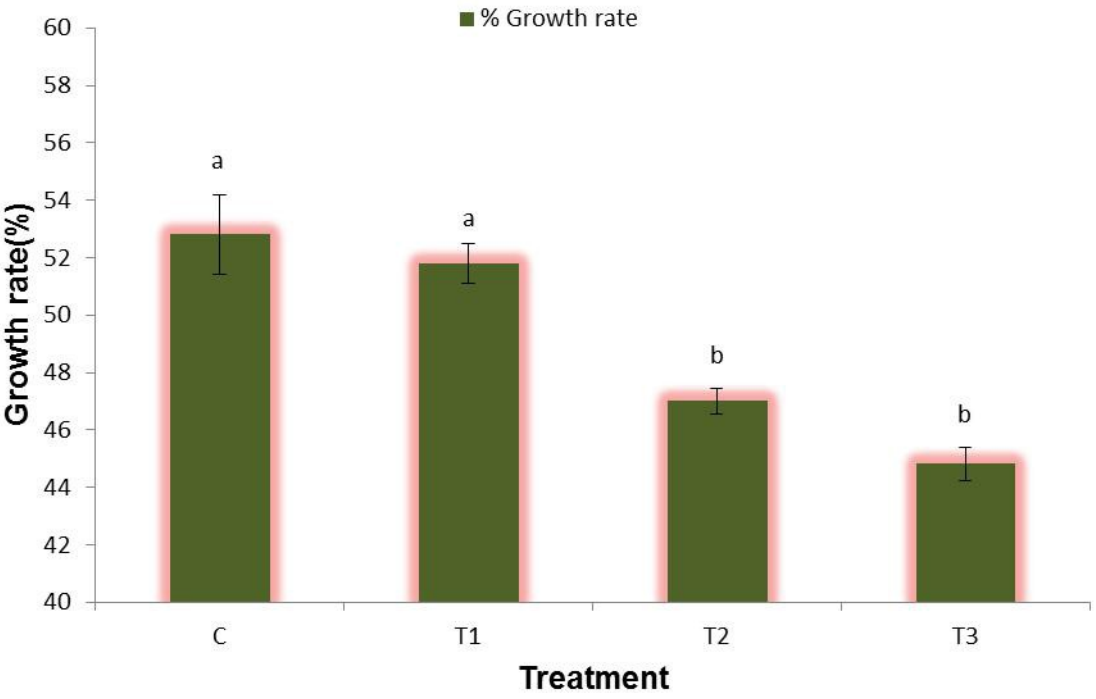

Fig. 2. Growth rate (%) of *C. magur* reared under different treatments

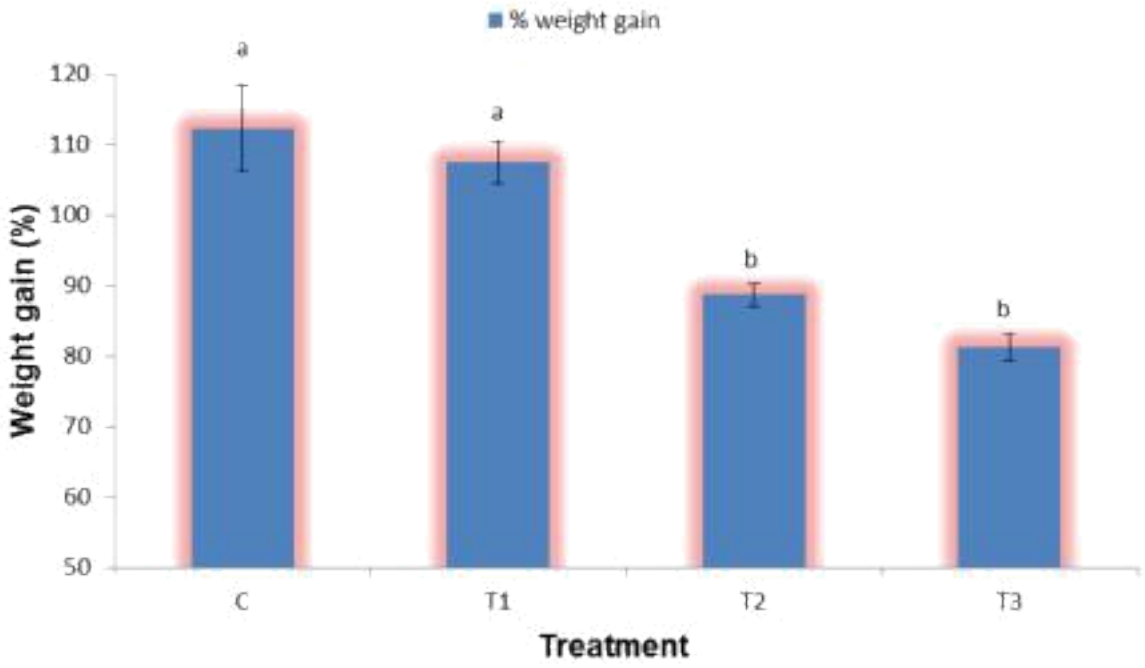

Fig. 3. Weight gain (%) of *C. magur* reared under different treatments

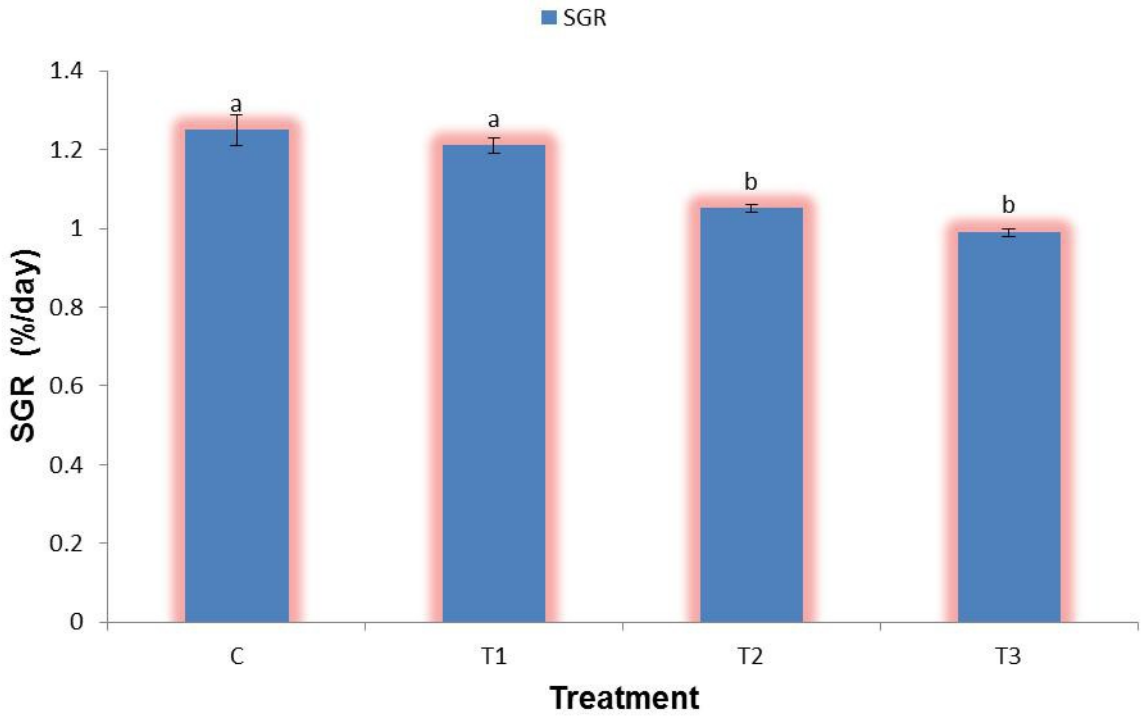

Fig. 4. SGR (%/day) of *C. magur* reared under different treatments

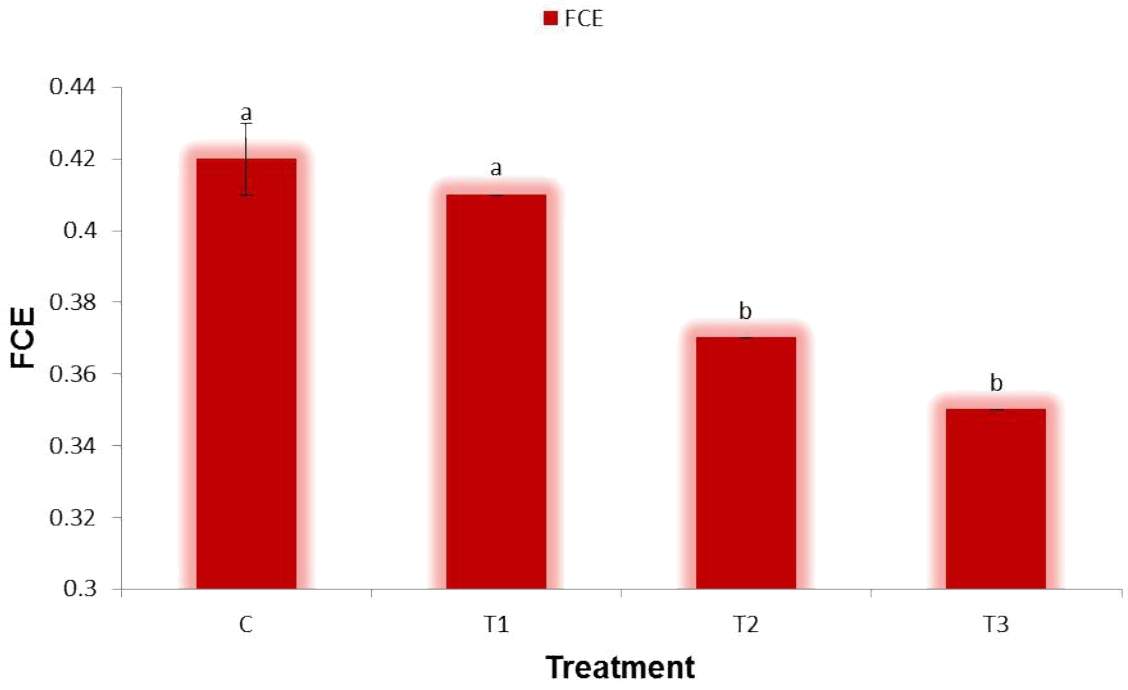

Fig. 5. FCE of *C. magur* reared under different treatments

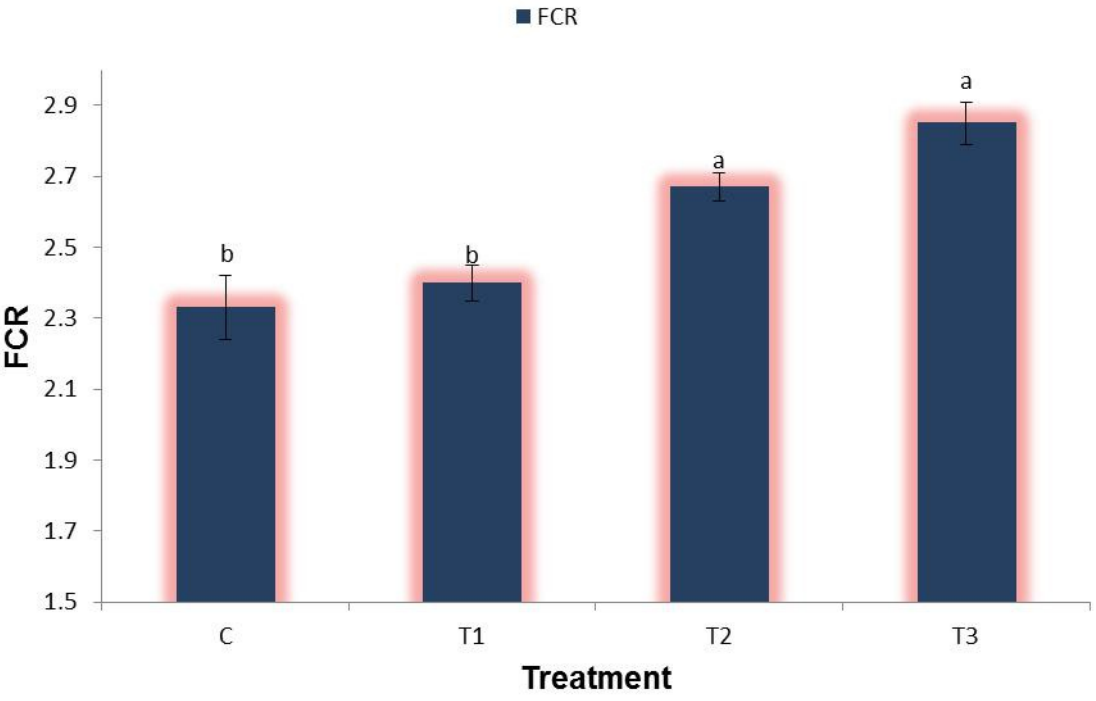

Fig. 6. FCR of *C. magur* reared under different treatments

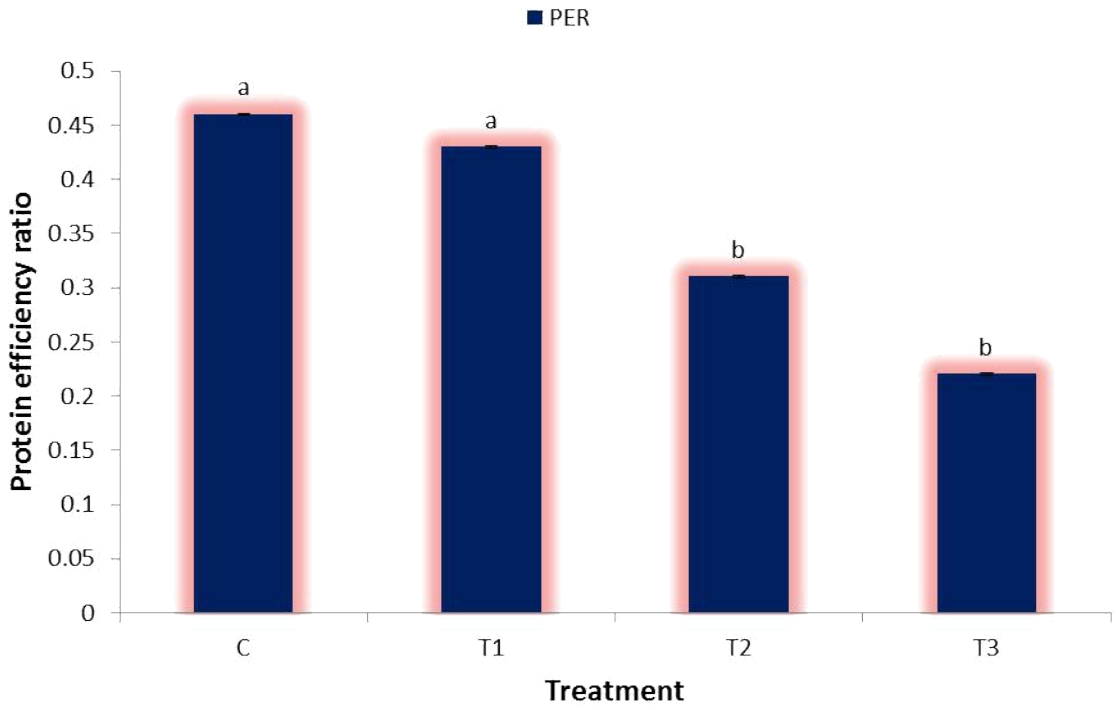

Fig. 7. Protein efficiency ratio of fishes reared under different experimental condition

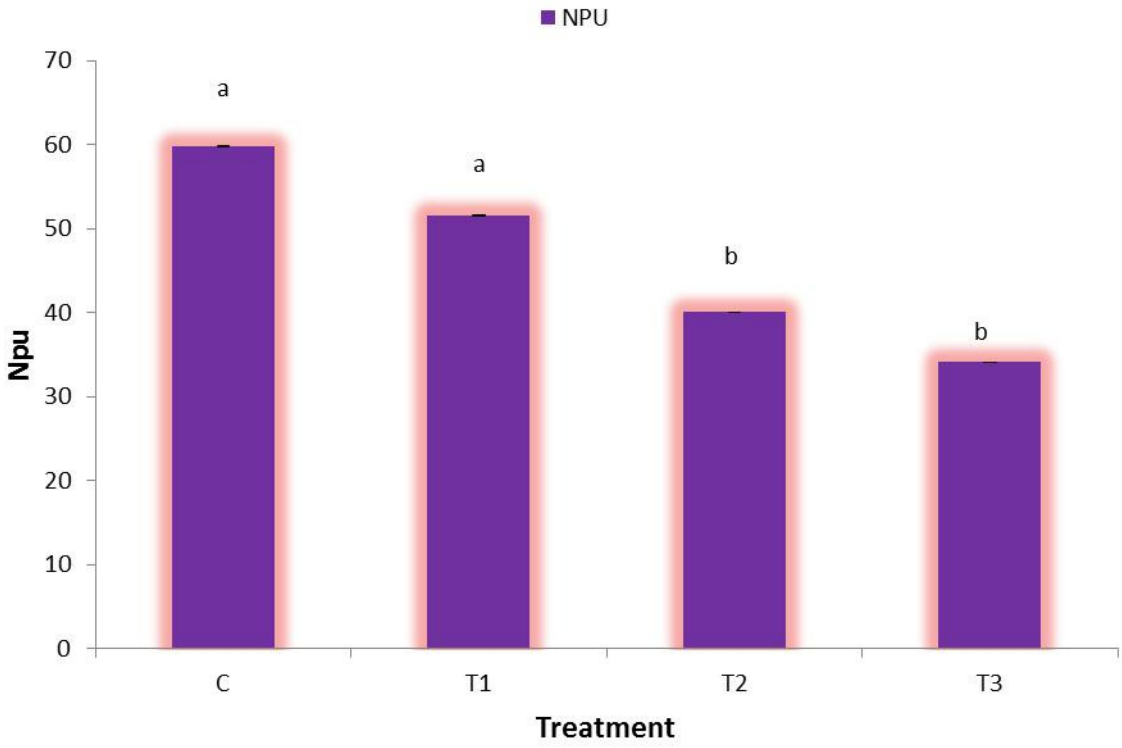

Fig. 8. NPU of fishes reared under different experimental condition

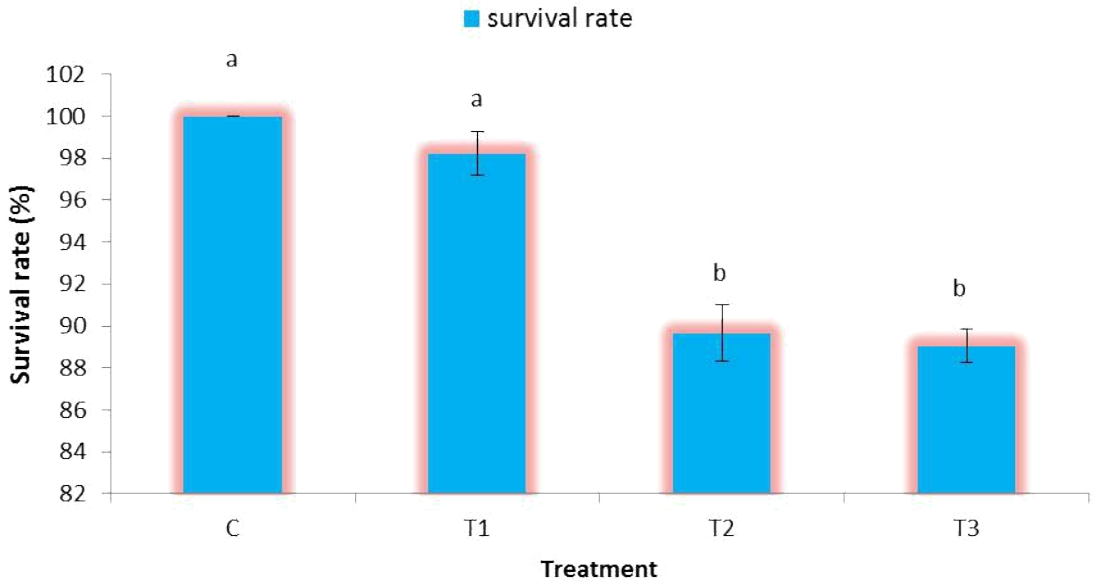

Fig. 9. Survival rate (%) of fishes reared under different experimental condition

4.3. Metabolic enzymes activity of *C. magur* reared under different experimental treatments

4.3.1. Alanine transaminase activity (ALT)

The ALT value observed in *C. magur* reared in different treatment groups is displayed in Table 9 and Fig.10. Lower level of ALT activity was observed in T3 (0.47 ± 0.12) and higher activity in T1 (1.41 ± 0.25), which was significantly different from other treatment groups.

4.3.2. Aspartate aminotransferase activity (AST)

The AST value acquired from the 60 days experiment is presented in Table 9 and Fig.11. The higher value of AST was exhibited by fish reared in control and T1. AST activity was significantly differed ($P<0.05$) among the different treatment groups.

4.3.3. Alkaline phosphatase (ALP)

The ALP value observed in *C. magur* in different treatment groups is displayed in Table 9 and Fig.12. Lower level of ALP activity was shown in T3 (0.63

± 0.02) and higher activity in T1 (1.04 ± 0.02), which was significantly different from other treatment groups.

Table 9: Metabolic enzyme activities of *C. magur* reared in different experimental treatments

Treatments	ALT (U/mg protein/min)	AST (U/mg protein/min)	ALP (U/mg protein/min)
C	1.57^{a} ± 0.18	2.37^{a} ± 0.08	1.06^{a} ± 0.02
T1	1.41^{a} ± 0.25	2.29^{a} ± 0.16	1.04^{a} ± 0.02
T2	0.69^{b} ± 0.07	1.62^{b} ± 0.03	0.76^{b} ± 0.05
T3	0.47^{b} ± 0.12	1.52^{b} ± 0.12	0.63^{c} ± 0.02

C=Control, T1= 10% inclusion of Jatropha, T2= 20% inclusion of Jatropha, T3= 30% inclusion of Jatropha. Values in the same column with different superscripts differ significantly (P<0.05) for each parameter. Data expressed as Mean ±SE, n = 3.

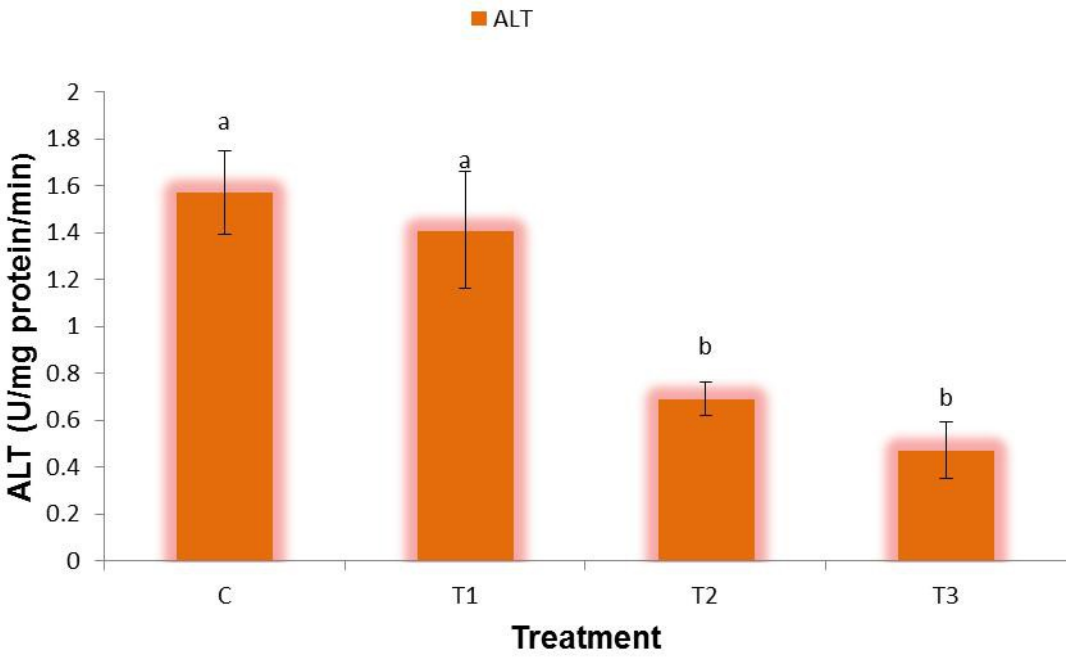

Fig. 10. ALT (U/mg protein/min) of *C. magur* reared under different treatments

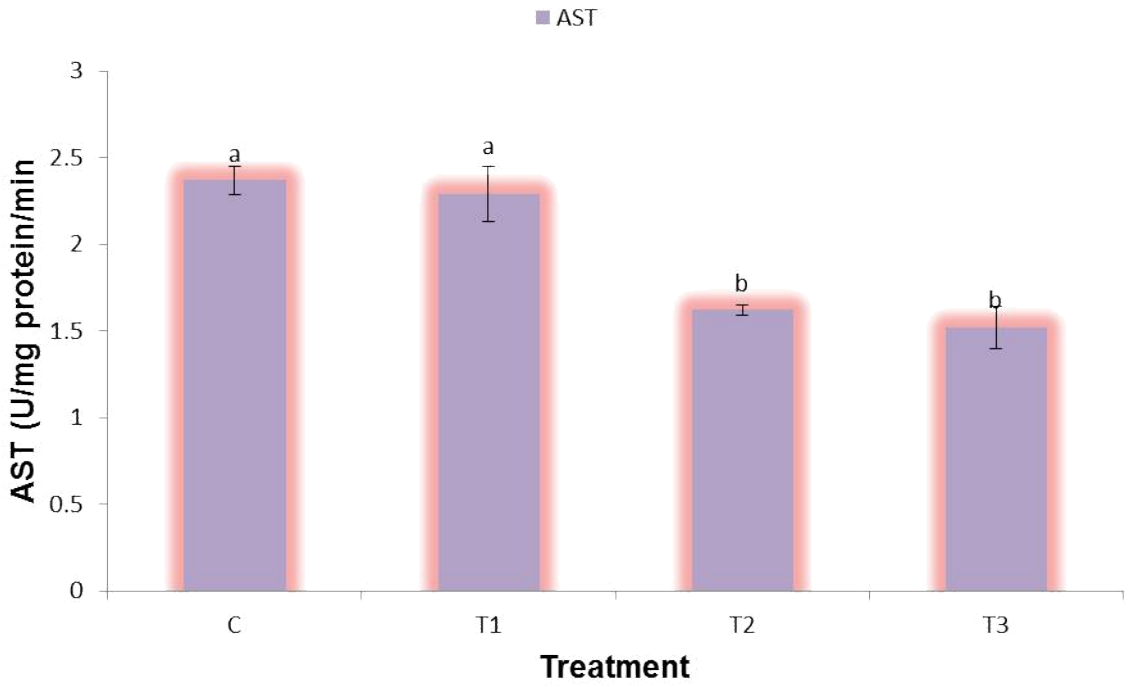

Fig. 11. AST (U/mg protein/min) of *C. magur* reared under different treatments

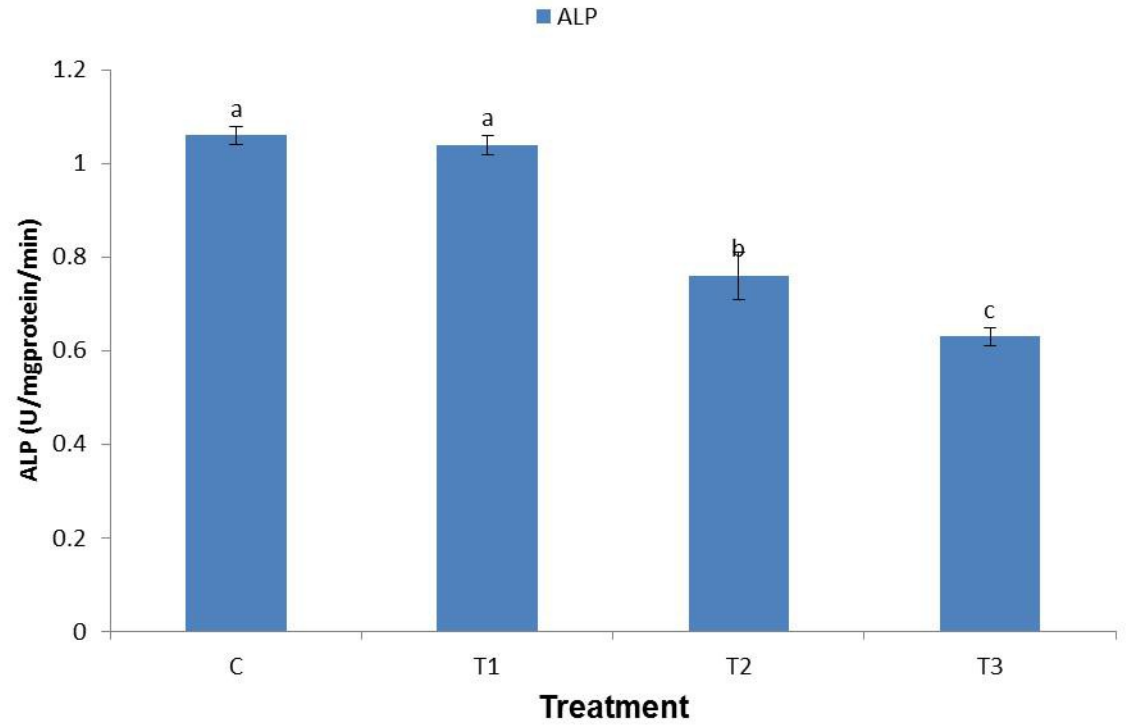

Fig. 12. ALP (U/mg protein/min) of *C. magur* reared under different treatments

4.4. Digestive enzymes activity of *C. magur* reared under different treatment groups

4.4.1. Protease

The protease values observed in *C. magur* at the end of the experiment is displayed in Table 10 and Fig. 13. Significantly lower protease activity was showed by T3 (0.14 ± 0.01) group raised fishes. Fish reared in control exhibited higher protease activity (0.35 ± 0.01) which was not significantly different from T1 group

4.4.2. Amylase

The amylase activity obtained at the end of 60 days experimental trail is shown in Table 10 and Fig. 14. Amylase activity was lowest in T3 (0.11 ± 0.01) reared fishes. Higher amylase activity was shown by fish reared in control (0.28 ± 0.01) which was not significantly different from T1group.

4.4.3. Lipase

The lipase activity obtained at the end of 60 days experimental trail is shown in Table 10 and Fig. 15. Lipase activity was lowest in T3 (3.99 ± 0.79) reared fishes. Whereas higher lipase activity was shown by fishes reared in control (value), which was not significantly different from T1 group.

Table 10: Digestive enzyme activity (U/mg protein/min) (Mean ± S.E) of *C. magur* reared under different experimental treatments

Treatment	Protease (U/mg protein/min)	Amylase (U/mg protein/min)	Lipase (U/mg protein/min)
C	0.40^{a} ± 0.02	0.30^{a} ± 0.01	7.22^{a} ± 0.90
T1	0.35^{ab} ± 0.01	0.28^{ab} ± 0.01	6.69^{ab} ± 0.95
T2	0.29^{b}± 0.01	0.24^{b} ± 0.01	5.52^{b} ± 0.02
T3	0.14^{c} ± 0.01	0.11^{c} ± 0.01	3.99^{c} ± 0.79

C=Control, T1= 10% inclusion of Jatropha, T2= 20% inclusion of Jatropha, T3= 30% inclusion of Jatropha. Values in the same column with different superscripts differ significantly (P<0.05) for each parameter. Data expressed as Mean ± SE, n = 3.

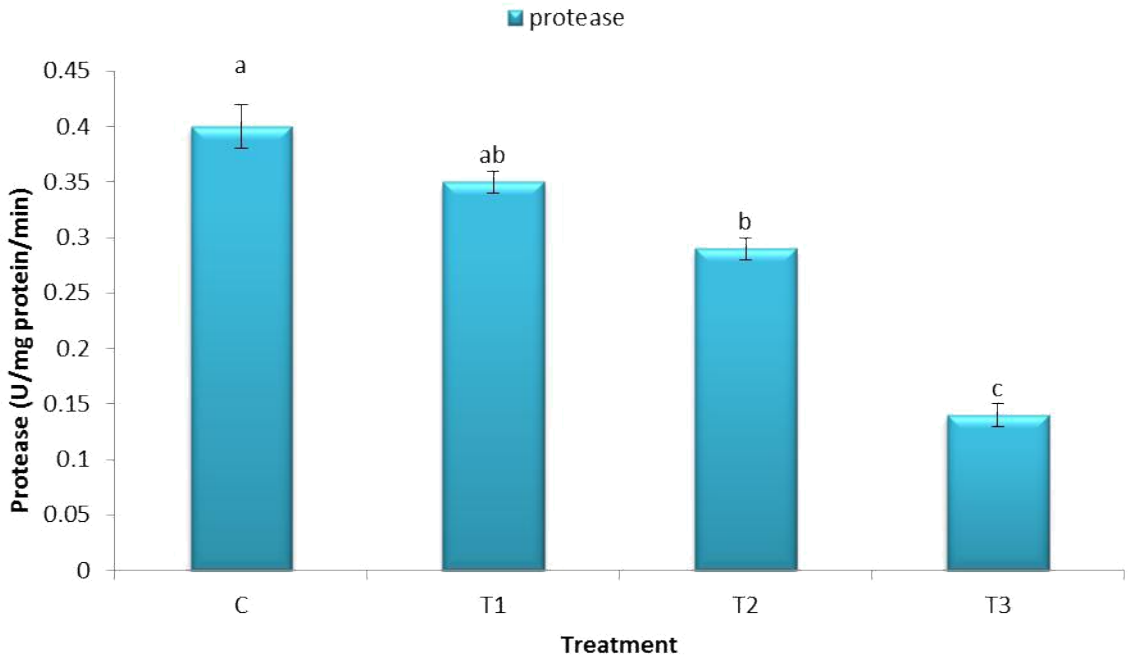

Fig. 13. Protease values (U/mg protein/min) of *C. magur* reared under different treatments

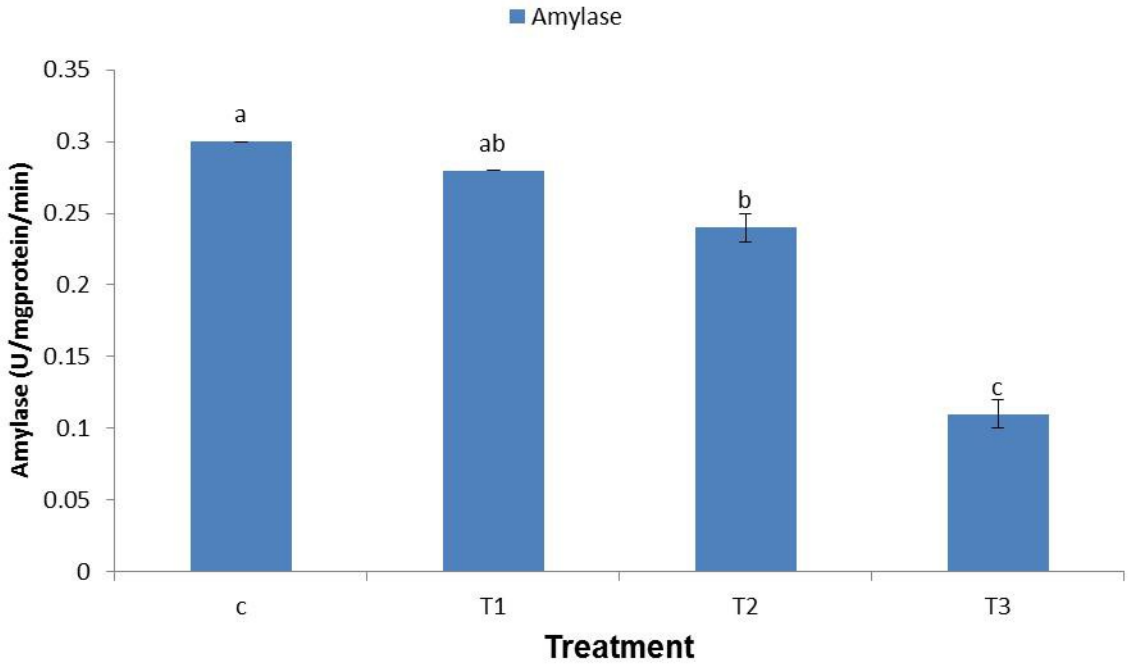

Fig. 14. Amylase (U/mg protein/min) of *C. magur* reared under different treatments

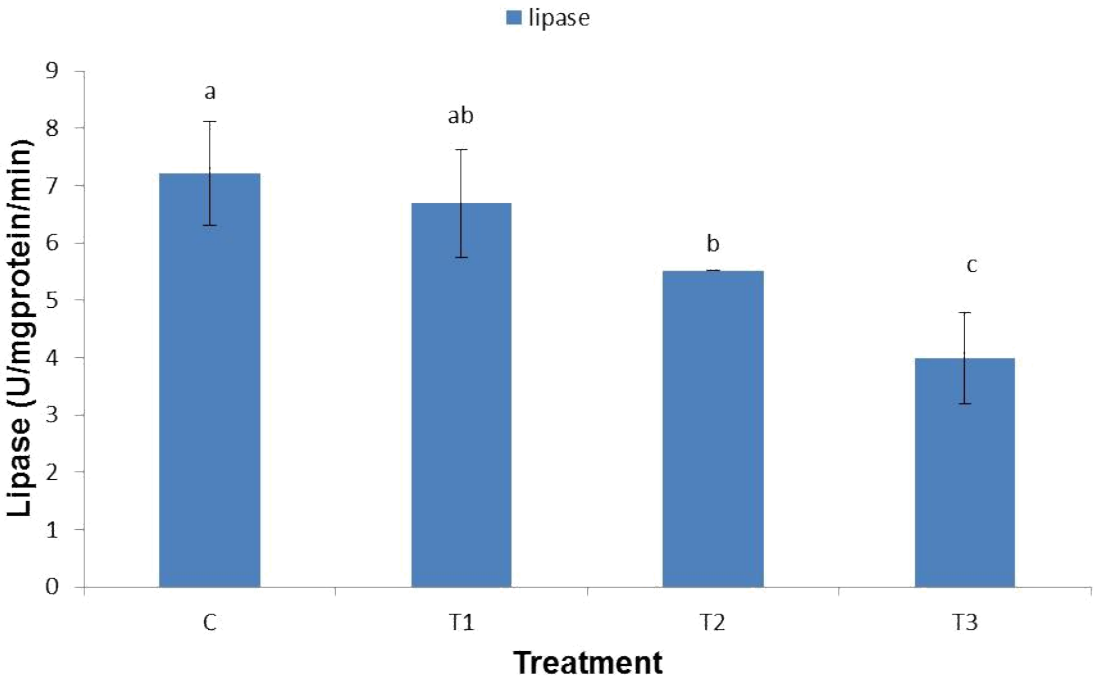

Fig.15. Lipase (U/mg protein/min) of *Clarias magur* reared under different treatments

4.5. Stress Parameters

4.5.1. Superoxide dismutase assay (SOD)

The superoxide dismutase value observed in *C. magur* at the end of the experiment is displayed in Table 11 and Fig. 16. Significantly lower SOD activity was showed by T1 (2.28 ± 0.03) group raised fishes. Fish reared in T3 exhibited higher SOD activity (3.40 ± 0.18) which was significantly different from T1 group.

4.5.2. Catalase assay

The Catalase value found in *C. magur* fed with experimental diet is displayed in Table 11 and Fig. 17. Significantly lower catalase activity was showed by T1 (1.57 ± 0.23) group raised fishes. Fish reared in T3 exhibited higher catalase activity (5.95 ± 0.32) which was significantly different from T1 group.

Table 11: SOD and Catalase Activity (U/mg protein) (Mean ± S.E) of *C. magur* reared under different experimental treatments

Treatments	SOD (Units/mg protein)	Catalase (Units/mg protein)
C	$2.23^{c} \pm 0.07$	$1.17^{b} \pm 0.13$
T1	$2.28^{c} \pm 0.03$	$1.57^{b} \pm 0.23$
T2	$2.97^{b} \pm 0.06$	$4.92^{a} \pm 0.87$
T3	$3.40^{a} \pm 0.18$	$5.95^{a} \pm 0.32$

C=Control, T1= 10% inclusion of Jatropha, T2= 20% inclusion of Jatropha, T3= 30% inclusion of Jatropha. Values in the same column with different superscripts differ significantly (P<0.05) for each parameter. Data expressed as Mean ± SE, n = 3.

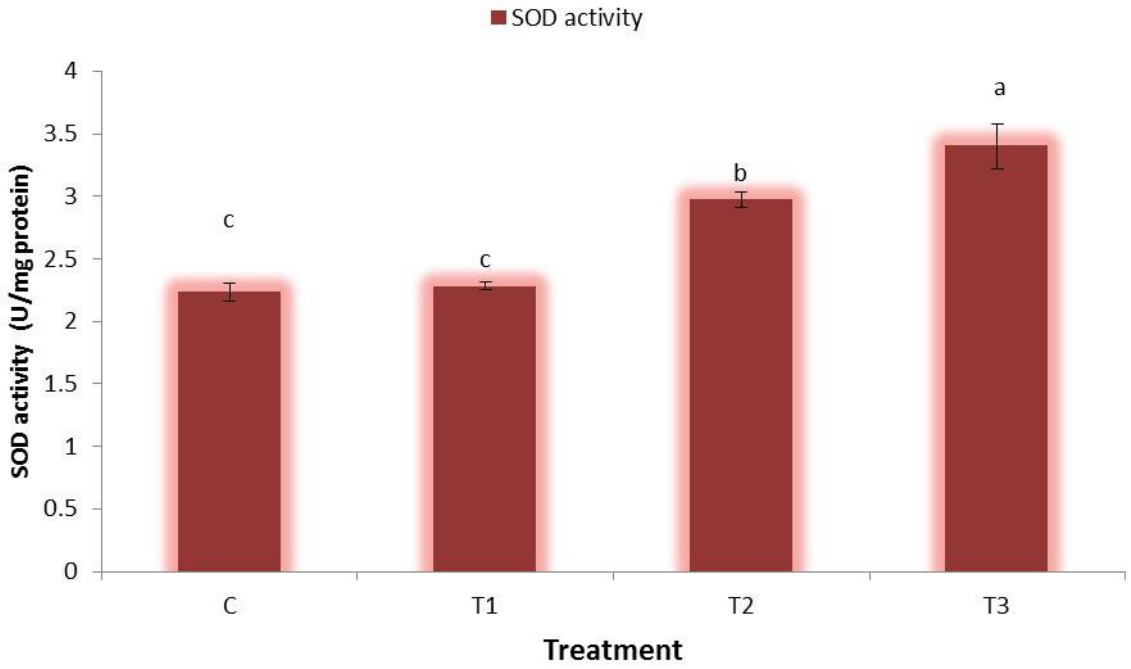

Fig. 16. SOD activity (U/mg protein) of *C. magur* reared under different treatments

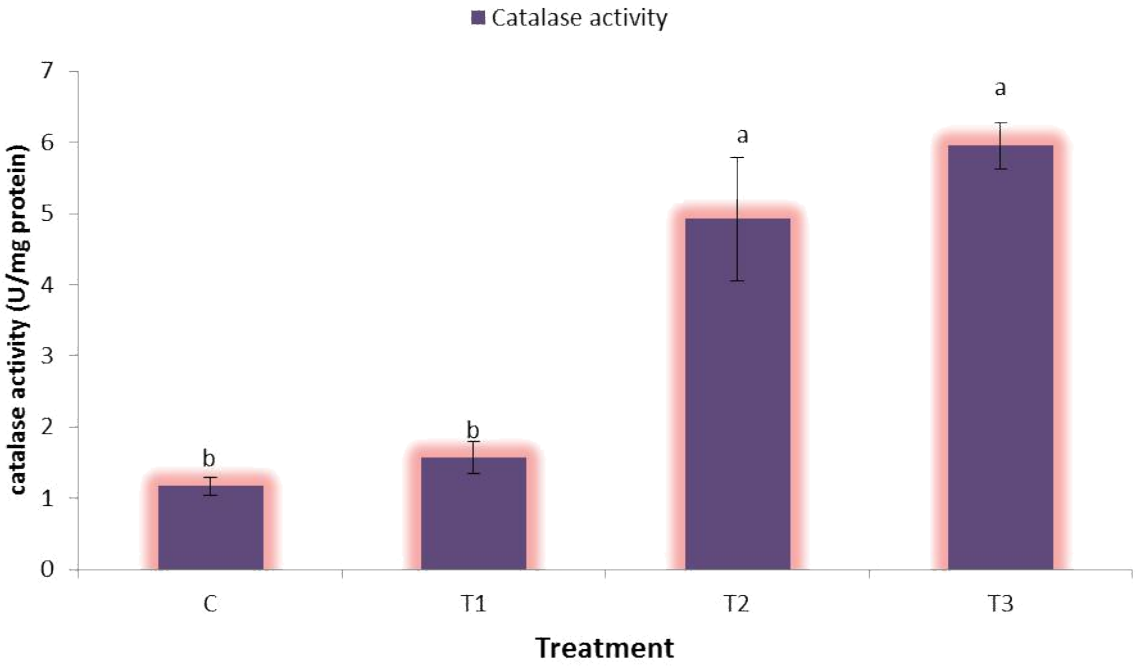

Fig. 17. Catalase activity (U/mg protein) of *C. magur* reared under different treatments

4.6. Whole body composition of the *C. magur* reared with different experimental diets

The whole body composition of the experimental fishes in terms of moisture, crude protein, crude lipid and crude fibre at the end of the experiment are given in Table 12.

4.6.1. Moisture

Higher moisture content was exhibited by fishes reared in T3 group, which is significantly different from other treatment groups (Fig. 18).

4.6.2. Crude protein (%)

The Crude protein (%) of the experimental fishes varied significantly ($P<0.05$) among the various treatment groups. The highest crude protein was recorded in control and T1 (Fig. 19).

4.6.3. Ether extract (EE)

The ether extract of the experimental fishes varied significantly (P<0.05) among the various treatment groups. The highest ether extract was found C and T1 group (Fig. 20).

4.6.4. Ash

The Ash content of the experimental fishes varied significantly (P<0.05) among the various treatment groups. The highest ash content was found in T3 group (Fig. 21).

4.6.5. Total carbohydrate

The Total carbohydrate content of the experimental fishes varied significantly (P<0.05) among the various treatment groups. The highest was found in T3 group (Fig. 22).

Table 12: Carcass Analysis(%) of *C. magur* reared under different experimental treatments

Treatments	Moisture	Crude protein	Crude lipid	Ash	TC
C	$74.81^{b} \pm 0.10$	$13.10^{a} \pm 0.10$	$4.50^{a} \pm 0.02$	$2.96^{b} \pm 0.22$	$4.63^{b} \pm 0.02$
T1	$74.44^{b} \pm 0.13$	$13.43^{a} \pm 0.17$	$4.38^{a} \pm 0.02$	$3.14^{ab} \pm 0.08$	$4.61^{b} \pm 0.04$
T2	$75.00^{a} \pm 0.16$	$11.49^{b} \pm 0.22$	$3.39^{b} \pm 0.02$	$4.22^{a} \pm 0.17$	$5.90^{a} \pm 0.05$
T3	$75.55^{a} \pm 0.22$	$11.29^{b} \pm 0.005$	$3.79^{b} \pm 0.03$	$4.30^{a} \pm 0.12$	$5.07^{a} \pm 0.07$

C=Control, T1= 10% inclusion of Jatropha, T2= 20% inclusion of Jatropha, T3= 30% inclusion of Jatropha. Values in the same column with different superscripts differ significantly (P<0.05) for each parameter. Data expressed as Mean ± SE, n = 3.

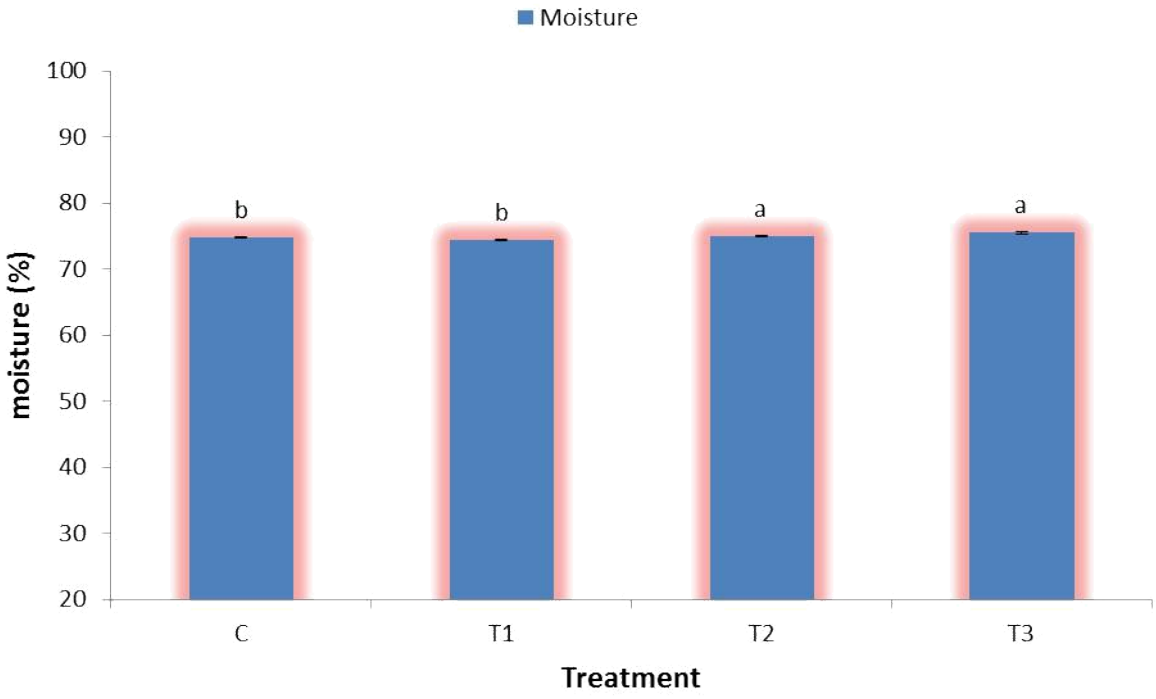

Fig. 18. Moisture content (%) in the whole body of *C. magur* reared with different experimental diets

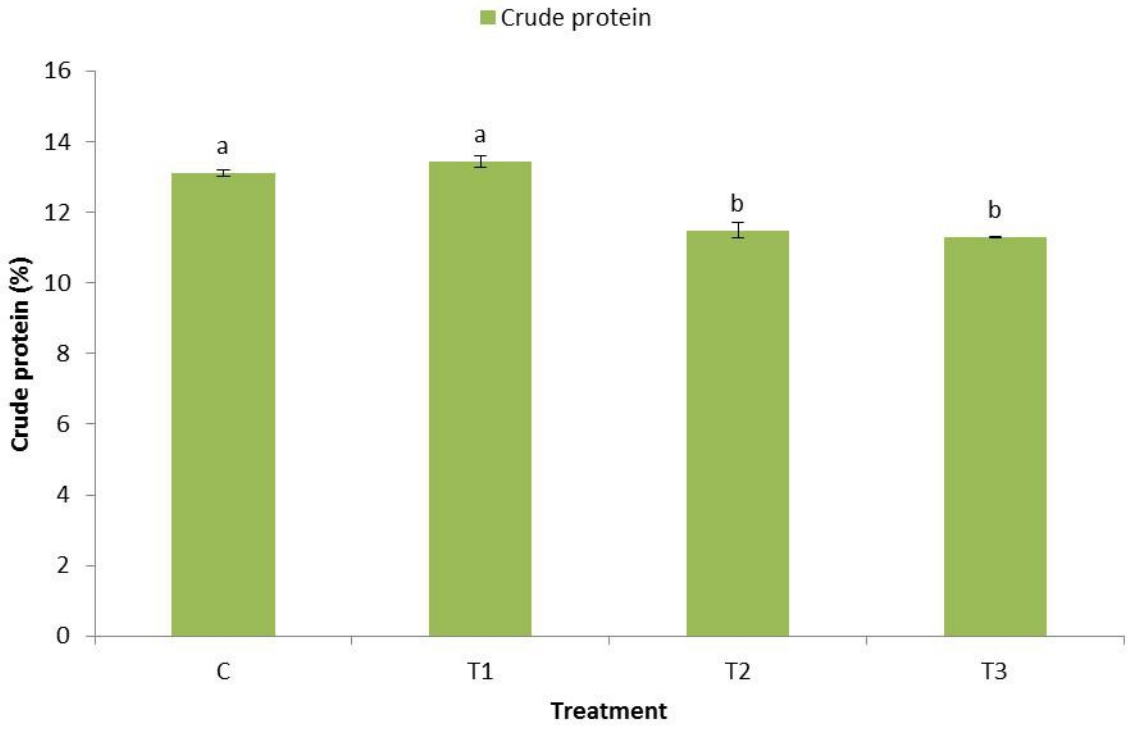

Fig. 19. Crude Protein (%) in the whole body of *C. magur* reared with different experimental diets

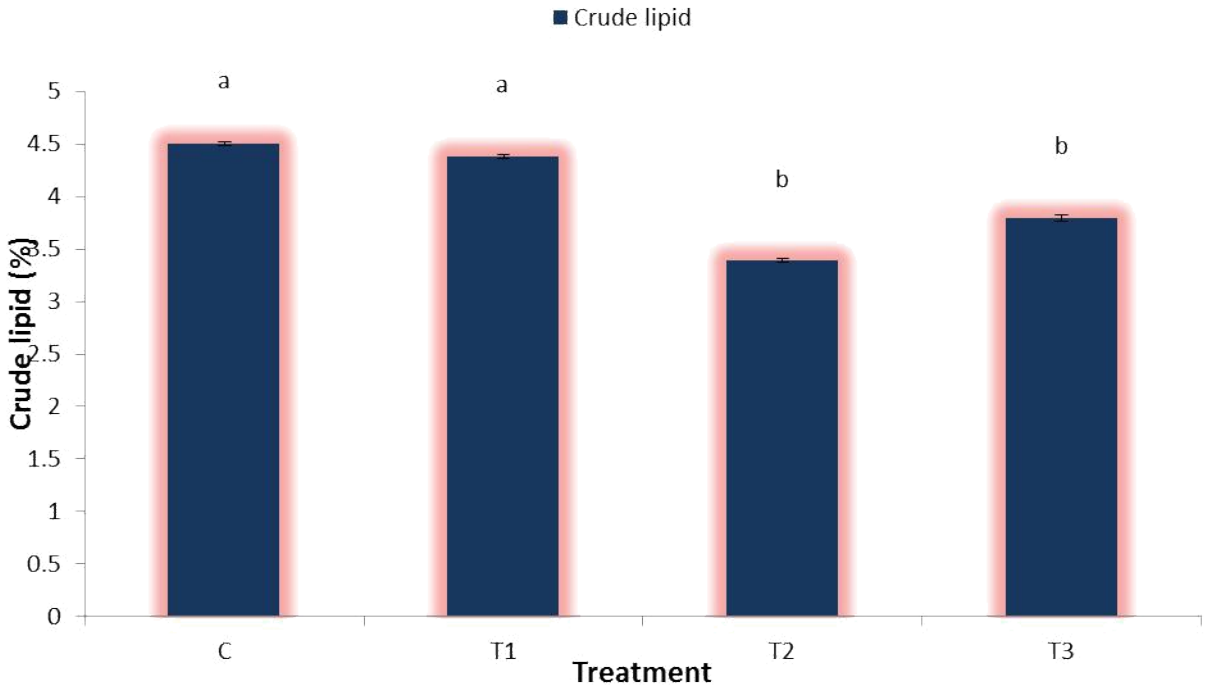

Fig. 20. Crude lipid (%) in the whole body of *C. magur* reared with different experimental diets

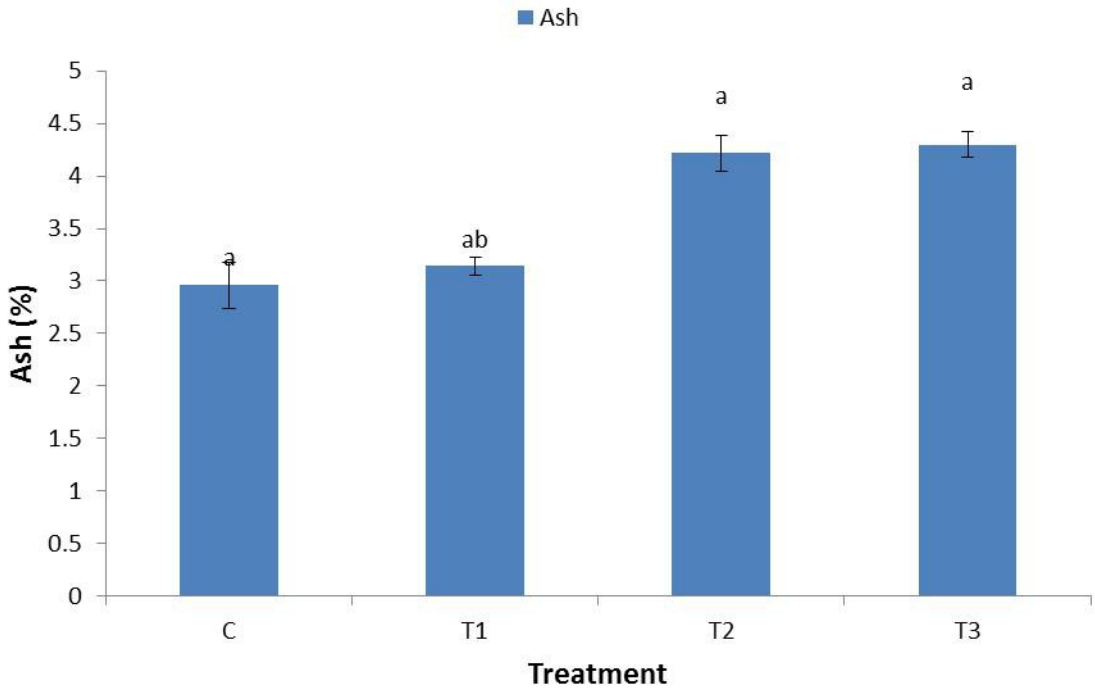

Fig. 21. Ash (%) in the whole body of *C. magur* reared with different experimental diets

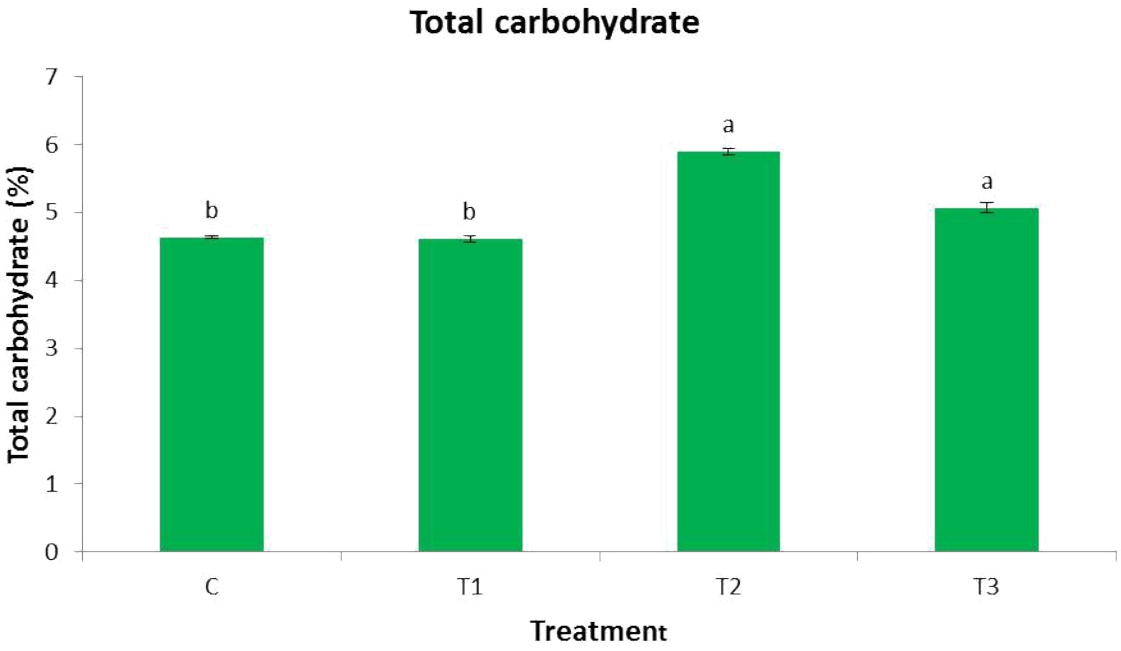

Fig. 22. Total carbohydrate (%) in the whole body of *C. magur* reared with different experimental diets

5. DISCUSSION

5.1. Water quality parameters

The water quality parameters observed during the experiment were within the suitable range for the rearing of *Clarias magur* fingerlings, indicating that the experimental condition was favoured the growth of this species (Bhatnagar and Devi, 2013).

Temperature observed in experimental tanks during the trial period varied within a range of 23 to 27.5ºC. The lowest temperature (23°C) was observed during mid (January-February, 2018) of the experiment due to the onset of the winter season at the experimental site. Temperature beyond the optimal value of a particular fish species will adversely influence the fish health by increasing oxygen consumption, metabolic rate and susceptibility to pathogens, which may create a variety of patho-physiological disturbances and can ultimately lead to the death of the species (Dalvi *et al.,* 2009). Pardue (1970) reported that lethal temperatures for *C. batrachus* range from 9.4-12.8°C. Dehadrai *et al.* (1985) observed that magur could grow well up to 32°C temperature and mortality started at 38°C. Hence, the recorded temperature ideally supported the growth of *Clarias magur* during the experiment. Magur being a hardy fish can tolerate acidic pH (but not less than 5) but shows better growth at neutral pH. Water pH varied throughout the experiment and found in the range of 7.0-8.4 which indicated that it was conducive for the growth of magur fingerling. Dissolved oxygen of water plays a vital role in water quality and during the experimental period it varied within 5.2-7.8 ppm. The air breathing catfish which needs less than 4 ppm DO for its survival and growth (Bhatnagar and Devi, 2013). Free carbon dioxide level in the water was negligible during the experiment due to alternate day siphoning of uneaten feed and faecal matters, water exchange and continuous aeration. The recommended water alkalinity and hardness for rearing of *Clarias sp.* were 90-160 ppm and 80-150 ppm respectively (Sahoo *et al., 2*016). During the experimental period, ground water was used to rear the fish and the recorded total alkalinity (150-162 ppm) and hardness (150-161ppm) were within the range to promote better fish growth. Ammonia-N, Nitrite-N and Nitrate-N

parameters were within the range as suggested by Boyd and Pillai (1984) and Rowland (1986).

5.2. Growth performance of *C. magur* fingerlings reared with different experimental diets

The *C. magur* fingerlings reared in control and T1 group showed better growth performance, in terms of average body weight and specific growth rate, compared to other treatments. The SGR result obtained in the present study was within the range as reported in *C. gariepinus* (Fakunle *et al.,* 2013). Kumar *et al.* (2011a) observed better growth performance of rainbow trout fed with detoxified *Jatropha* kernel meal at 34.3% inclusion level but the present study found better growth performance in 10% inclusion level. Lowest weight gain and SGR were observed in T3 which clearly indicates that increase in the inclusion level of detoxified *Jatropha* kernel meal in the diet will lead to the decreased growth of the fish due to the presence of anti-nutritional factors such as tannin and total phenol. Significantly lower growth performance of the fish reared in T2 and T3 might be due to poor digestibility of energy and protein in the diets (Kumar *et al.,* 2011a) resulting from lower protein quality and availability of energy from detoxified *Jatropha* kernel meal. According to Kumar *et al.* (2010) lysine is deficit in *Jatropha* kernel meal but there was no external supplementation of lysine in the the present study. In case of the control group and T1 soybean meal and fishmeal were the source of lysine but as the soybean meal was gradually replaced by the *Jatropha* kernel meal, lysine became the limiting growth factor. The presence of anti-nutritional factors in diet, after detoxification, might have affected the efficient utilization of feed in T2 and T3 groups. Initially, the fish exhibited slower growth due to the onset of winter, a drop in temperature (23-24°C), and it was picked up in the mid of the experiment.

FCR and FER values are used as an important parameter for determining growth, as feed constitute the largest operational cost in aquaculture system (Amin *et al.,* 2005). Fish with lowest FCR and highest FER is a prime element to produce a healthy product in an economically viable way. Ndome *et al.* (2011) stated that most of the fish shows good growth when the FCR value is within a range of 1.5-2.0.

The FCR value observed in the present experiment was lowest in control and T1 fed group, which indicates that less quantity of feed is required by the fish to gain body flesh. Solomon *et al.* (2016) also observed that carnivorous fish *C. gariepinus* fed with a diet containing detoxified *Jatropha* kernel meal at 13% inclusion level was having lowest FCR compare to higher inclusion level.

The present study revealed that 20% and 30% inclusion of detoxified *Jatropha* kernel meal resulted in an increase in FCR and reduction in FER. Fish cannot exhibit similar kind of growth (in weight) for the same amount of feed (in weight) given because it might spend more energy for digestion, stress mechanism and other metabolic purpose (Ndome *et al.,* 2011). The reason behind lower FCR and higher FER in the fish reared in control and T1, might be due to the availability of more quantity of good quality protein in the diet.

PER is a measure to know how efficiently fish can utilize the protein present in the feed (Nalawade and Bhilave, 2011). Protein is the most expensive ingredient in aqua feed, excess protein in the feed will increase operational cost (Ahmad *et al.,* 2005). Efficient protein synthesis needs sufficient bioavailability of all essential amino acids (Dabrowski and Guderly 2002). The present study showed higher PER and NPU value in the control and T1 group which clearly indicated that 10% inclusion of detoxified *Jatropha* kernel meal did not reduce the bioavialability of quality protein, which can be further correlated with the digestive enzyme protease activity. Kumar *et al.* (2011a) reported that, comparatively, the plant protein source DJKM has more complex protein structure than the SBM. So the decrease in the PER and NPU values, in higher inclusion level, may be due to the presence of more complex structure of detoxified *Jatropha* kernel meal protein in diet, which makes the digestion difficult for the fish.

The present study found that survival rate of *C. magur* was higher in control and T1 group but comparatively lower in T2 and T3 group. Poor survivability of the fish in T2 and T3 may be due to the stress caused by the presence of heat stable anti- nutritional factor, tannin and total phenol.

5.3. Digestive enzymes activity of *C. magur* fingerlings fed with different experimental diets

Bolasina *et al.* (2007) mentioned that digestive enzyme activity was assessed to know the digestive status and nutritional condition of fish. The improvement in growth performance of fish in terms of nutrient utilization is a reflection of the better digestion which is influenced by digestive enzyme activity (Lemieux *et al.,* 1999). The present study results showed that fish reared in control and T1 exhibited a significantly higher amylase, protease and lipase activity whereas T2 and T3 showed a significantly lower digestive enzyme activity. A similar result was observed by Kumar *et al.* (2011a) in rainbow trout. The reason might be the presence of increased level of anti-nutritional factor in the diet with the increased inclusion level of detoxified *Jatropha* kernel meal. Some heat stable anti-nutritional factors, has the ability to inhibit the activities of the digestive enzymes such as trypsin, pepsin and alpha-amylase (Robaina *et al.,* 1995;). In other way, phytate can form a complex with proteins (Moyano *et al.,* 1999) and minerals (Sugiura *et al.,* 1999), which modify digestion processes by hindering the intestinal absorption. Similar results were reported by Sandholm *et al.* (1976), and Krogdahl *et al.* (1994), they reported that protease enzyme (for example trypsin) activity decreases with the increase in the inclusion level of plant protein in trout diet. Further, they found that digestive enzyme activity is highly sensitive to plant anti-nutrients.

Cheng *et al.* (2004) recommended that for plant-based diet, phytase has to be added in the feed at a level of 500 FTU kg^{-1} to neutralise the effect of the anti-nutritional factor, phytate. However, in the present study, tannin and total phenol were responsible for lower protease activity in the higher inclusion level of detoxified *Jatropha* kernel meal.

Increased activity of digestive enzyme might have helped in better digestion of the feed in control and T1. Because the increased digestive enzyme activity, improve the fish ability to digest major nutrients (lipid, protein & carbohydrate) and increase the efficient feed utilization.

5.4. Metabolic enzymes activity of *C. magur* fingerlings fed with different experimental diets

Kumar *et al.* (2011a) observed normal organ function on feeding of Detoxified *Jatropha* Kernel Meal to rainbow trout. The present study revealed that control and T1 group had a higher amount of metabolic enzymes activity in liver tissue which clearly indicated that increase in inclusion level of *Jatropha* kernel meal cause reduction in the AST, ALT and ALP level. ALT, AST and ALP function as a link between protein and carbohydrate metabolism and help to optimize growth and energy utilization (Shamna *et al.,* 2015). AST and ALT catalyse the compounds, like α- ketoglutarate and alanine to pyruvic acid and glutamic acid, responsible for the synthesis of non-essential amino acids which in turn help in protein synthesis and improve the growth of fish. (Aprajita *et al.,* 2017). Phulia *et al.,* 2017 reported increased level of AST and ALT activities in the liver and muscle of fish fed 200 and 300 g kg^{-1} FJKM. The increased accumulation of toxic compounds in organs i.e. liver and kidney, may inhibit the metabolic enzyme activity (Sampath *et al.,* 2002). Reduction in the metabolic enzyme activity with the increased inclusion level of detoxified *Jatropha* kernel meal was due to the presence of anti-nutritional factors which leads to damage of liver tissue and resulting in the reduction of metabolic enzyme activity.

5.5. Stress Enzymes

SOD and Catalase are important antioxidant defence mechanism which avoids oxidative stress by hunting the reactive oxygen species (ROS) and thus helps to sustain homeostasis (Shin *et al.,* 2010). SOD enzyme dismutase or reduce the ROS compounds like superoxide anion (O^{2-}) into toxic H_2O_2 which further catalysed into water and oxygen by catalase enzyme (Vera-Jimenez *et al.,* 2013).

The change in oxidative defence system of fish is used as a biomarker during pro-oxidant situation, such as nutritional deficiency, which reduces growth and immunity (Morales *et al.,* 2004). In fish, nutrient deprivation could augment the production of free radicals and H_2O_2 as a consequence an elevated activity of SOD and catalase were observed to avoid oxidative stress (Mohapatra *et al.,* 2017). In

the present study higher SOD and catalase activity were found in T3 and showed lower activity in T1. The reason might be the presence of anti-nutritional factor in the diet which created oxidative stress to the fish.

5.6. Whole body composition of *C. magur* fingerlings reared with different experimental diets

The study on biochemical changes in carcass tissue are useful to assess the energy metabolism rate and depletion of reserved energy which are considered as a good indicator of energy requirement study (Jobling and Davies,1980). The changes in body composition is observed as a biochemical response of fish to a stress (De Coen and Janssen, 1997). The composition and quality of diet used in rearing period influences the body composition (Jobling *et al.,* 1994). Proximate composition of the magur fingerings fed with T1 diet showed more protein retention in the body at the end of the experiment. This indicated that the protein to energy ratio used in the feed was at the right proportion (Solomon *et al.,* 2016) also observed more protein content in *Clarias gariepinus* tissue fed with diet containing 13% of *Jatropha* kernel meal. Better protein utilization by the fish of control and T1 group leading to more protein accretion in the fish muscle and attributing to better body composition.

5.7. Economic Analysis

Considering the price of feed ingredients, cost of control,T1, T2, and T3 feeds are Rs. 45.5, 30.48, 26.48, and 22.48 respectively. The cost of T3 feed is less compare to other treatments. However, based on growth performance and cost effectiveness the economic analysis results of present study revealed that 10% inclusion level of DJKM (T1 diet) is economically viable.

6. SUMMARY

Aquaculture is an important animal protein producing sector for increasing global population. As a result of this, global demand for protein rich feed ingredients for the production of animal protein is increasing. So research is needed to find an alternative source of protein to meet the growing demand of protein based feed ingredients for the production of quality aquafeed for sustainable aquaculture practices.

The present work was conducted to study the effect of detoxified *Jatropha* kernel meal (DJKM) inclusion on growth and physiological changes of *Clarias magur* fingerlings. The study was conducted using a Completely Randomized Design (CRD), where each of the treatments was triplicated. The experiment consisted of three treatments (T1, T2, and T3) and a control. 0% inclusion of DJKM was used as a control group and 10%, 20% and 30% inclusion were used as treatments T1, T2, and T3 respectively. The study was conducted for 60 days where each tank was stocked with 20 fish with an average body weight of 5 ± 0.5 g. Feed was given twice a day (@ 5% body weight), during morning and evening at 07.00 hrs and 17.00 hrs respectively.

Length and weight of the fish and water quality parameters like temperature, pH, dissolved oxygen, ammonia, nitrite, total hardness and total alkalinity were observed after 15 days of interval.

Growth parameters obtained from the present study clearly showed that fishes reared in control and T1 group exhibited higher growth rate compared to fishes raised in T2 and T3 fed group. The lowest FCR and higher FER, PER and survival rate were observed in control followed by T1. However, there is no significant difference ($p>0.05$) between control and T1. The experiment revealed that *C. magur* showed reasonably good growth up to 10% DJKM inclusion in the diet but further increase in the inclusion level negatively affects the growth of this species. Digestive enzyme and metabolic enzyme activity were also found to be higher in control and T1 fed group compare to other treatments. Reduction in the

enzymatic activity with the increased inclusion level of DJKM was due to the presence of anti-nutritional factors which leads to damage of muscle tissue and liver.

The present study revealed higher SOD and catalase activity in T3 and showed lower activity in T1. The reason might be due to the presence of anti-nutritional factors in the diet which created oxidative stress to the fish.

Proximate composition of the magur fingerings fed with T1 diet showed more protein retention in the body at the end of the experiment. This indicated that the protein to energy ratio used in the feed was at the right proportion. Hence, considering growth performance and cost effectiveness of the diet T1 (10% inclusion of DJKM) is the best treatment. So, it can be concluded that *C. magur* can tolerate 10 % inclusion of DJKM in its diet without affecting the growth.

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ANNEXURE

ANOVA	Analysis of Variance
Cm	Centimetre
DO	Dissolved Oxygen
EDTA	Ethylenediamine tetraacetic acid
FAO	Food and Agriculture Organization
%	Percentage
G	Gram
ha	Hectare
Kcal	Kilo Calorie
Kg	Kilo Gram
L	Litre
mg	Milli Gram
Min	Minutes
ml	Milli Litre
mM	Milli Molar
ppm	Parts Per Million
rpm	Revolution Per Minute
U	Units
°C	Degree Centigrade
µg	Micro Gram
SBM	Soybean meal
FM	Fish meal
DJKM	Detoxified *Jatropha* kernel meal