



## Effect of fish meal substitution with lima bean meal on growth and feed utilization in common carp fry, *Cyprinus carpio*

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### ABSTRACT

An experiment was conducted in an earthen pond in the west region of Cameroon to investigate the level of replacing fish meal (FM) with lima bean meal (LBM) on zootechnic parameters in common carp fry. Four hundred and fifty fish weighing  $0.91 \pm 0.03$  g were divided into five groups of 90 fish each. In control group (LBM-0), fish received normal feed, while in four experimental groups, FM was replaced with LBM at 10%, 20%, 30% and 40% corresponding to LBM-10, LBM-20, LBM-30 and LBM-40. Fish were fed three times daily for 84 days. Every 14 days, fish were harvested for weight determination. The results obtained showed significant increases in weight gain (WG) and specific growth rate (SGR). After 84 days of feeding, fish fed in LBM-40 showed a WG of  $6.47 \pm 0.48$  g and SGR of  $2.70 \pm 0.11\%/d$  compared to LBM-0 (WG= $3.52 \pm 0.17$  g and SGR= $1.95 \pm 0.08\%/d$ ), corresponding to a significant increases of 58.27% and 27.77% respectively. Concerning feed utilisation, LBM-10 produced the greatest effect with the lowest feed conversion ratio (FCR= $0.71 \pm 0.05$ ) and the highest protein efficiency ratio (PER= $3.54 \pm 0.26$ ) compared to LBM-0 (FCR= $1.09 \pm 0.12$  and PER= $2.36 \pm 0.25$ ). These results showed that FM substitution with LBM improves growth and feed utilization in *Cyprinus carpio* fry.

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**Keywords:** *Cyprinus carpio*, fish meal, lima bean, growth, feed utilization.

### INTRODUCTION

World aquaculture is developing fast enough to overcome the increase in fish demand for human consumption, while the amount of captured fish is declining (Reverter et al., 2014; Hixson, 2014). This development

is due to the level of intensification of fish culture in both the developing and industrialized countries. Aquaculture requires optimization of nutrition to efficiently raise fish for the purpose of food production. Fishmeal, being an excellent nutrient profile, is a major

source of animal protein generally used for all types of fish such as Common carp (*Cyprinus carpio*), which is one of the most popular freshwater species in aquaculture worldwide (FAO, 2007 ; Hasan et al., 2007; Njoukou et al., 2013; Mehdi et al., 2016). Most of the high dietary protein requirements of fish are best supplied by fish meal (FM) because of its nutritional value and palatability. However, increasing fishmeal cost and competition with other livestock feed industries are the main factors that hinder the sustainable development of aquaculture in most developing countries. So, identification and evaluation of alternative protein sources to fish meal with cheaply and abundantly available for use in fish formulation is a top research priority in fish nutrition (Watanabe, 2002; Köprücü and Sertel, 2011; Gan et al., 2017). Considerable attention has been devoted to the evaluation of plant protein such as soy bean meal, lupin meals, various legumes (cow pea, green mung bean, rice bran) and leaf meals (Ramachandran and Ray, 2007; Latif et al., 2008; Lim and Lee, 2008; Tahir et al., 2008; Sivani et al., 2013) as ingredients in feeds of aquatic animals. The present study was undertaken to evaluate the possibility of utilizing lima bean meal in diets for the common carp *Cyprinus carpio* by assessment of its growth response to varying levels of lima bean meal in the diet.

## MATERIALS AND METHODS

### Preparation of lima bean meal

Lima bean seeds used for this research work were purchased from a local market in Batié, in the West region of Cameroon. A set of lima bean (200 g) were soaked in water, in a seed to water ratio of 3:10 (kg/L). Eleven hours after, the soaked beans were removed from water and immediately boiled in water at 100 °C for 3 h; with the water being changed after every one hour (Adeparusi and Ajayi, 2004;

Falaye et al., 2014). After boiling, the water was drained off and the boiled seeds were sun dried and ground into fine powder, stored in air tight container and kept at room temperature until used.

### Feed ingredients and diet formulation

Five isonitrogenous diets were formulated at 40% protein. A control diet (LBM-0) contained 24.42% fish meal as the primary source of animal protein without the LBM, and this was compared with test diets LBM-10, LBM-20, LBM-30 and LBM-40, where lima bean meal was added approximately at 10–40% in the test diets to substitute fish meal. The levels of other ingredients were adjusted to maintain the same dietary protein when LBM was incorporated at 10, 20, 30 and 40%. The vitamin, mineral mix and cassava flour were kept constant in all diets while soybean oil and palm oil as lipid sources were mixed at a ratio of 1:1 as shown in Table 1. All ingredients were milled separately to fine particulates, weighed, mixed except the cassava flour and they were blended manually. Cassava flour was gelatinized and cooked in 600 ml water, allowed to cool and added to the mixture. The resulting mixture was then passed through the meat pelletizer to obtain 2 mm pellet. The “spaghetti-like” strands were sun dried, placed in plastic bags and stored at room temperature until used.

The chemical composition of the test diets were analysed by standard methods (AOAC, 1990). Moisture was analysed by drying the sample in an air convection oven at 105 °C overnight. Crude protein was analysed by the Kjeldhal method after acid digestion (% crude protein = % nitrogen x 6.25); crude lipid after extraction with petroleum ether by the Soxhlet method. The ash contents in the diets were analysed by combustion of samples in a muffle furnace at 550 °C for 12 h (Table 1).

### Experimental fish

The study was carried out between August and October 2015, in a private fish farm at Batié (LN: 5°17'0''-5°18'53'' and LE: 10°17'0''-10°19'31'') in the West Region of Cameroon (Njoukou et al., 2013; Zango et al., 2016). A total of 450 *Cyprinus carpio* fry weighing  $0.97 \pm 0.03$  g were obtained from the semi-artificial breeding in the same private fish farm and acclimatized in 6000 L rectangular cement tank for 3 days prior to the experiment. During the acclimation, the fish were fed LBM-0 control diet three times per day (06:00 am, 12:00 noon and 18:00 pm).

### Experimental design

For the purpose of this study, the common carps fry randomly selected were distributed in 15 net cages ( $0.5 \times 1 \times 1$  m<sup>3</sup>, mesh size of 1.5 mm) installed in a cement tank ( $3 \times 2 \times 1.2$  m<sup>3</sup>) and supplied with borehole water with continuous aeration by using air pumps to ensure proper oxygenation. For each feed i.e. control (LBM-0) and test feeds (LBM-10, LBM-20, LBM-30 and LBM-40), three replicates were used and in each replicate 30 fish were stocked. They were fed at the rate of 10% of live net weight three times in a day for 84 days. Every 14 days, the fish were weighed and the feed amounts were adjusted according to the new weights.

Water quality parameters (temperature, dissolved oxygen, pH, nitrate, nitrite, total hardness, carbonate hardness and Chlorine) were measured every day before feeding. Water temperature (T °C) was measured using a Maximum-minimum thermometer; dissolved oxygen (O<sub>2</sub>) was determined using JBL Test Kits while pH, nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), total hardness (GH), carbonate hardness (KH) and Chlorine (CL<sub>2</sub>) were measured using Test strips (JBL Easy Test 6in1)

### Measurement of growth parameters

The growth performance was assessed by determination of feed intake (FI), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate as described by Todorov and Ivancheva. (1992) as follows:

1. Weight Gain (WG) = final weight – initial weight
2. Daily Weight Gain (DWG) = Mean weight gain / T, where T is the number of days in the experimental period
3. Specific growth rate (SGR) =  $100 (\ln W_2 - \ln W_1) / T$ , where W<sub>1</sub> and W<sub>2</sub> are the initial and final weight, respectively, and T is the number of days in the experimental period
4. % Survival rate = final number of fish  $\times$  100 / initial number of fish
5. Feed efficiency ratio (FER) = weight / feed intake
6. Feed conversion ratio (FCR) = feed intake / wet weight gain, where feed intake = feed distributed – feed not consumed
7. Protein efficiency ratio (PER) = wet weight gain / protein fed, where Protein fed = % protein in diet  $\times$  total diet consumed / 100

### Statistical analysis

All results were expressed as mean  $\pm$  SD. The data collected during every fish sampling were analyzed by two-way analysis of variance (ANOVA-2) followed by Bonferroni multiple range test with n=10 per replicate, while data collected at the end of the experiment were subjected to one-way analysis of variance followed by Duncan's multiple Range Test with n=3 replicates. Differences were regarded as significant when  $P < 0.05$ ; All statistics were carried out using *GraphPad Prism* version 5.0.

**Table 1:** Composition and proximate analyses of the experimental diets used during the study (g/100 g dry weight).

Ingredients	Control diet		Experimental diets		
	LBM-0	LBM-10	LBM-20	LBM-30	LBM-40
Fish Meal	24.42	21.98	19.53	17.31	14.65
Lima Bean Meal	//	6.48	12.95	19.30	25.47
Soybean cake	24.42	25.22	26.23	27	28.30
Cotton seed cake	24.42	25.22	26.23	27	28.30
Palm cake	7.24	5.36	3.35	1.62	//
maize meal	7.24	5.36	3.35	1.62	//
Wheat bran	7.24	5.36	3.35	1.62	//
Cassava flour	1	1	1	1	1
Soybean oil	1	1	1	1	1
Palm Oil	1	1	1	1	1
Vitamin mix	1	1	1	1	1
Mineral mix	1	1	1	1	1
<b>Proximate composition (%)</b>					
Dry matter	86.83	88.86	88.80	88.93	87.80
Crude protein	39.05	39.97	40.06	39.03	39.02
Crude lipid	10.84	7.71	10.62	8.03	12.75
Carbohydrates	22.7	21.7	23.6	25.9	26.3
Crude ash	10.42	13.11	14.38	16.85	19.59
Energy (kcal/100g)	393.9	391.5	464.6	375.1	486.8

**RESULTS**

**Environmental conditions during the growth test**

Water physico-chemical parameters during the experiment are presented in Table 2. It can be noticed that the mean value of temperature, dissolved oxygen, pH, nitrite, nitrate, total hardness, carbonate hardness and Chlorine remained within the optimum for the common carp breeding. The total hardness (5±3.02 dGH) and carbonate hardness (3.75±1.35 dKH) were below the range of 8-12 and 5-12, respectively for total hardness and carbonate hardness.

**Growth performances and diet utilization**

Growth parameters recorded during fish sampling showed an increase in weight gain with respect to time but not according to the level of fish meal substitution. At the sixth fish sampling, the weight gain of the carp from both the control and experimental groups were respectively LBM-0 (3.52±0.17 g), LBM-10 (4.94±0.29 g) LBM-20 (5.66±0.70 g), LBM-30 (5.55±0.66 g) and LBM-40 (6.47±0.48 g); corresponding respectively to significant

increase (p<0.05) of 28.74%, 37.81%, 36.57% and 45.60% as compared to the control (Figure 1A). The substituted diets induced significant increase (p<0.05) in specific growth rate all over the experimental period compared to the control (Figure 1B).

The 14<sup>th</sup> day feed consumption response is shown in Figure 2. Independently to the type of feed fed, feed intake increased gradually in all groups with time. However, on the 84<sup>th</sup> day of the experiment, the feed intake by *Cyprinus carpio* fry as recorded from LBM-40 (156.74±6.18 g) was the highest (p<0.05) while that in LBM-10 (136.60±3.50 g) was the lowest (P>0.05) as compared to LBM-0 (131.79±1.92 g).

**Production parameters of *Cyprinus carpio* fry fed various replacement levels of lima bean meal**

Table 3 shows the parameters observed and recorded in the survival, growth performance and nutrient utilization of *Cyprinus carpio* fry fed with replacement levels (10%, 20%, 30% and 40%) of lima bean meal in the diets.

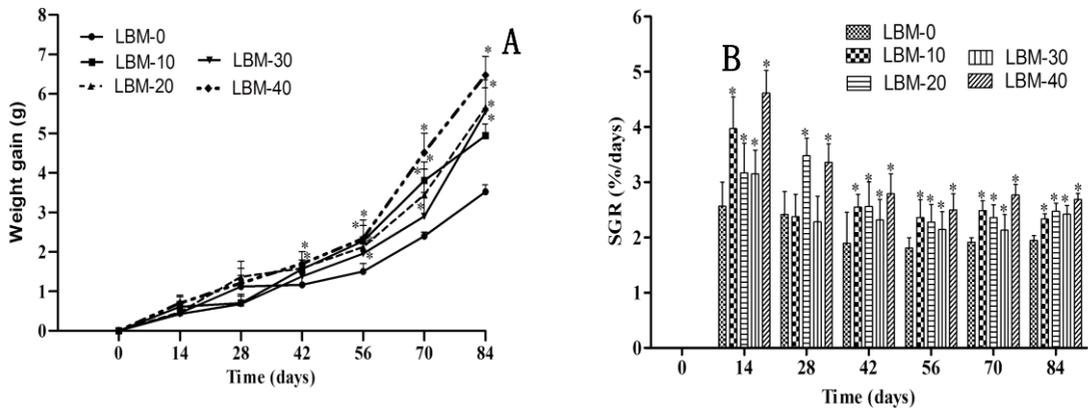
The survival rate across the treatments were not significantly different ( $p>0.05$ ). LBM-40 had the highest survival rate 100% and the lowest was obtained in LBM-10 which is 91%.

Both the mean weight gain (MWG) and average daily weight gain (ADWG) across the treatments were significantly different ( $p<0.05$ ). LBM-10 had the highest values of MWG (193.4 g) and ADWG (2.3 g/d) while the lowest values obtained in the experimental groups was shown in diet LBM-20 the values of MWG and ADWG being respectively 143.6 g and 1.71 g/d. though the highest value of the average specific growth rate was obtained with LBM-10 (2.51%/d), no significant different was observed as compared with that obtained with the normal diet LBM-0 (1.98%/d).

Both the total feed intake (TFI) and total protein intake (TPI) observed in treatments showed that all formulated diets were nutritionally adequate. However, both the TFI and TPI were significantly different in the experimental group compared to the control. Among the experimental diets, LBM-40 had the highest values of both the TFI (156.74 g) and TPI (61.16 g). The lowest values of TFI (136.60 g) and TPI (54.60 g) were recorded for diet LBM-10. The neither food conversion ratio (FCR) nor protein efficiency ratio (PER) in experimental diets was not significantly different compared to the control diet. However, fry fed with diet LBM-10 showed the lowest value of FCR (0.71) and the highest value of PER (3.54) followed by diet LBM-30; with values of FCR and PER that were 0.93 and 2.75 respectively.

**Table 2:** Water physico-chemical parameters in the net cages during the experiment with common carp fry.

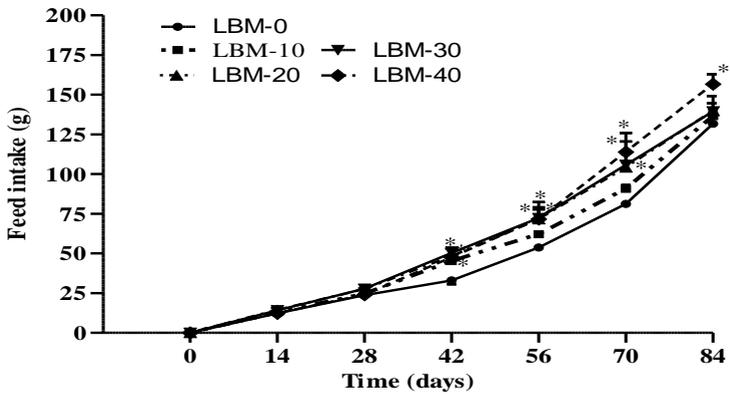
parameter	Value obtained	optimum values (Morin, 2012 ; Zaykov and Staykov, 2013)
temperature, °C	22.1±0.72	22-26
dissolved oxygen, mg.l <sup>-1</sup>	8.66±0.98	>5
pH	6.5±0.18	6.5-8.5
nitrite, mg.l <sup>-1</sup>	0.1±0.02	<0.2
nitrate, mg.l <sup>-1</sup>	7.5±4.52	<25
total hardness, °dGH	5±3.02	8-12
carbonate hardness, °dKH	3.75±1.35	5-12
Chlorine, mg.l <sup>-1</sup>	0	0



**Figure 1:** Growth performance of *Cyprinus carpio* fry fed on experimental diets for 84 days.

Values are means ± standard deviation of three replications containing ten fish per replication.

\* $p<0.05$ , significantly different compared to LBM-0.



**Figure 2:** Feed intake in *Cyprinus carpio* fry fed on experimental diets for 84 days. Values are means ± standard deviation of three replications containing thirty fish per replication. \*p<0.05, significantly different compared to LBM-0.

**Table 3:** Survival, Growth and feed utilization of *Cyprinus carpio* fry fed different diets for 84 days.

Parameter	Control diet		Experimental diets		
	LBM-0	LBM-10	LBM-20	LBM-30	LBM-40
IN	90	90	90	90	90
FN	83	81	87	89	90
SR (%)	93±1.92	91±1.11	97±1.92	99±1.11	100±0.00
IB (g)	28.51±3.88	27.10±4.54	27.93±0.44	28.17±0.78	26.78±3.90
FB (g)	150.07±12.27	220.5±15.5*	171.53±21.17	177.27±7.38*	192.4±3.32*
MWG (g)	121.56±12.17	193.4±19.17*	143.6±20.73	149.1±7.04*	165.61±10.78*
ADWG (g/d)	1.44±0.14	2.3±0.22*	1.71±0.24	1.77±0.08	1.97±0.12*
ASGR (%/d)	1.98±0.16	2.51±0.28	2.15±0.12	2.2±0.04	2.36±0.21
TFI (g)	131.79±1.92	136.60±3.50	139.85±5.58	139.57±4.5	156.74±6.18*
TPI (g)	51.46±0.76	54.60±1.40	56.02±1.60	54.43±4.25	61.16±2.41*
FCR	1.09±0.12	0.71±0.05	0.99±0.33	0.93±0.10	0.95±0.07
PER	2.36±0.25	3.54±0.26	2.68±0.76	2.75±0.29	2.71±0.22

Values are means ± standard deviation of three replications containing thirty fish per replication. \*p<0.05, significantly different compared to LBM-0.

IN, initial number; FN, final number; SR, survival rate; IB, initial biomass; FB, final biomass; MWG, mean weight gain; ADWG, average daily weight gain; ASGR, average specific growth rate; TFI, total feed intake; TPI, total protein intake; FCR, food conversion ratio; PER, protein efficiency ratio.

**DISCUSSION**

Fish meal is the main source of protein in commercial aquafeeds because it is known to contain complete essential or indispensable amino acids profile that is needed to meet the protein requirements of most fish species (Abowei and Ekubo, 2011; Ambani, 2015; Mouhamadou and Cheikh, 2015). The high

cost and fluctuating quality of fish meal have prompted the need to search alternative protein sources which are cheaply and abundantly available for use in fish formulation (Olsen and Hansen, 2012; Gan et al., 2017). The use of nonconventional feed stuffs of plant origin has been reported with good growth and better cost benefit values (Sogbesan et al., 2006).

Nowadays, partial or total replacement of protein in fish meal with plant sources has been an area of focus in aquaculture nutrition research. Lima bean (*Phaseolus lunatus*) has been previously studied as an alternative protein source in fish diet and seems to be a promising protein source (Adeparusi and Ajayi, 2004; Falaye et al., 2014; Ndidi et al., 2014). The results of the present study indicated that water quality parameters (temperature, dissolved oxygen, pH, nitrites and nitrates) throughout the experimental period were within the optimal range of common carp production (Zaykov and Staykov, 2013). These results showed that water quality did not produce any stress on fishes during experiment. Some mortality observed in both the control group (LBM-0=7%) and the experimental groups (LBM-10=9%, LBM-20=3% and LBM-30=1%) could not be attributed neither to the water quality nor to the diet; since most of the mortalities were observed one or two days after fish sampling, therefore they could be largely due to stress induced during the fish sampling. The results on both the growth and nutritional performances demonstrated the positive influence of the test diets in common carp fry. Throughout the experimental period, feed intake was high among the plant substituted diets compared to the normal diet. A lot of evidence has been presented that fishes are especially sensitive to the quality of feed and the presence of dietary anti-nutritional factors (Shakya, 2017). One of the most commonly encountered problems with alternative vegetable protein sources in aquaculture feeding is the palatability of fish. The results of the present study on feed intake indicated that the experimental diets might be more palatable than the normal diet. Although growth increase in plant substituted diets was independent to the level of substitution, both the weight gain and specific growth rate were significantly high among the plant substituted diets compared to the normal diet. These results indicate that common carp fry have successfully valorised nutrients from the lima bean meal. These results are in agreement with those obtained by Yuangsoi and Masumoto (2012), Sivani et al.

(2013) and Staykov et al. (2015) who found that the partial substitution of plant sources in fish diets improved growth performance of common carp (*Cyprinus carpio*). It is known that the utilization of nonconventional foodstuffs of plant origin needed improvements in proximate composition and antinutritional factors analyses since the presence of these antinutritional factors at higher inclusion levels is known to negate growth and other physiological activities (Oresegun and Alegbeleye, 2001; Ferouz et al., 2012). According to many authors, heat treatment has been shown to improve dietary utilization in legumes by modifying the fiber structure (Alonso et al., 2000; Drew et al., 2007; Vodouhe et al., 2012). Moreover, Falaye et al (2014) while studying the apparent digestibility coefficient of differently processed Lima Bean (*Phaseolus lunatus*) for *Clarias gariepinus* juveniles have demonstrated that boiled lima bean had the highest protein digestibility coefficient. So, good growth performances observed in the experimental diets fed groups could be partially attributed to the lima bean meal protein digestibility and assimilability due to the increase in gland stimulation and the action of digestive enzymes. It is known that Lysine, methionine, tryptophan, and arginine are the limiting essential amino acids which determine the growth and development of common carp (Antanasoff, 2014). Moreover, the protein in fishmeal is an excellent source of the aforementioned amino acids lysine, methionine and tryptophan in fish feeds (Pike and Barlow, 2003). Also, Ezeagu and Ibegbu. (2010) while studying the biochemical composition and nutritional potential of ukpa: a variety of tropical lima beans (*phaseolus lunatus*) from Nigeria revealed high essential amino acids contents. Accordingly, the helpful effects induced by the experimental diets fed groups on fish growth performances might be attributed to the combine action of these essential amino acids present in both the lima bean meal and fish meal. The results of the present work showed that the alternative protein source is well utilized by the common carp fry, because the experimental diets fed

groups showed significant improvement in survival, weight gain, specific growth rate and nutritional indices such as feed conversion ratio and protein efficiency ratio. In the present study, the values of the feed conversion ratio of lima beans meal substituted groups were comparatively lower than in the control group with the protein efficiency ratio comparatively higher in the test groups than in the control group. This indicates that partial substitution of fish meal with lima bean meal could improve the profitability of common carp farming by shortening the production cycle and a use of less quantity of food to a great production.

### Conclusion

The study clearly demonstrated that partial substitution of fish meal can be done by utilizing lima bean meal as an ingredient in diets of common carp. Partial substitution of fish meal with lima bean meal influenced positively the growth, did not have a negative impact on fish survival, increased the weight gain and specific growth rate, reduced the feed conversion ratio and improved the protein efficiency ratio. The present experiment showed that lima bean meal could be successfully used for replacement of fish meal in common carp feeds.

### COMPETING INTERESTS

The authors declare that there is no competing interests regarding the publication of this paper.

### AUTHORS' CONTRIBUTIONS

PN and JDD contributed to the supervision of the work. PNM was the principal investigator. METT and FT provided technical advices. The five authors also contributed to the manuscript preparation and correction.

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