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Effect of Various Levels of Raw *Citrullus lanatus* Seed Meal Diets on Growth Performance of *Cyprinus carpio* Fingerlings

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Abstract

The nutritional value of feeding *Cyprinus carpio* various levels of raw watermelon seed meal was evaluated in this study. Five diets of 35% crude protein were formulated with different levels of raw seed meal at 0%, 5%, 10%, 15% and 20% inclusion. Twenty fingerlings were randomly allocated in triplicate for each treatment in 70 liters plastic bowls, aeration was provided to culture bowls throughout the 12-week trial period. The results revealed that the inclusion level of 10% raw watermelon seed meal in the diet gave the best mean weight gain, feed conversion ratio, feed conversion efficiency, protein efficiency ratio, apparent net protein utilization, specific growth rate and survival rate. The growth performance decreased with increasing the inclusion level of raw seed meal beyond 10% ($P<0.05$). The inclusion level of 10% raw watermelon seed meal in the diet of common carp is found to be ideal for enhancing growth and better nutrient utilization.

Key words: Watermelon Seed, Unconventional Feed Stuff, Common Carp.

1. Introduction

Common carp is the most extensively cultivated freshwater fish species in the world (Komen, 1990; Zhou *et al.*, 2004). This fish has several advantages that make it popular for commercial culture: very fast growth rate, high environment tolerance, ease of handling, ability to be raised in high density, ability to utilize artificial diet with a relatively low protein content, and occurrence of highly productive strains and breeds produced from long-term domestication and selective breeding (Kirpitchnikov, 1999). Common carp is a largely benthic species that prefers shallow water habitats covered with aquatic weeds and grasses. It is an omnivorous fish that mostly feeds on the bottom but can exploit all levels in the water column. The natural diet of carp is dominated by chironomids, snails, young clams, shrimps and other benthic animals. This species also consumes aquatic plants, filamentous algae, seeds of plants and organic detritus. Under pond culture conditions, common carp will take soybean and peanut cakes, rice and wheat bran (Christopher *et al.*, 2007).

The importance of artificial feeding in aquaculture cannot be over-emphasized. It promotes faster growth, allows higher stocking density and shorter cultivation periods. However, the unavailability and affordability of adequate fish feed has significantly affected the development of aquaculture in Nigeria. A well prepared and carefully formulated fish feed plays a significant role in fish culture (Ukagha, 2003). The higher cost and competition imposed on some feed ingredients, such as soybean, groundnut cake, maize and sorghum used by human population as food, have necessitated the use of unconventional material for fish feed formulation. The price of finished feed in our part of the world continues to be on the rise, thereby removing the margin of profit accruing to the fish producers (Amubode and Ogogo, 1994). Thus, overcoming the burden of feed ingredients and reducing the cost of fish feed and fish products have been the burden of numerous researchers (Ani and Okeke, 2003). In fish farming, nutrition is critical because feed represents 40-50% of the production cost (Steven, 2001).

Watermelon belongs to the genus *Citrullus* and family *Cucurbitaceae* (Huxley, 1992). The *Cucurbitaceae* is a medium sized plant family, primarily found in warmer regions of the world. Watermelon seeds are a source of protein, B vitamins, minerals (such as magnesium, potassium, phosphorus, sodium, iron, zinc, magnesium and copper) and fat, among others (Collins *et al.*, 2007; Vandermark, 2011). Thus, the consumption of a variety of plant foods, including watermelon seeds, may provide additional health benefits (Cutter, 2000). The quality of the protein was moderate with methionine and cysteine as limiting amino acids, with supplementation of the deficit amino acids watermelon seeds can form a good source of protein. The chemical composition suggests its suitability as a matrix for mineral fortification and its functionality suggests that the watermelon seed is suitable for food formulations (Lakshmi and Kaul, 2011). This study

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attempts to evaluate the watermelon seed's nutritional value as an unconventional feed source, a byproduct protein and a lipid source in the diet of *Cyprinus carpio.*

2. Materials and Methods

2.1. Experimental Site

The feeding trial was carried out at the fish farm of Bauchi State Agricultural Development Program (BSADP) Bauchi, Nigeria. A circular plastic bowls system of 70 liters capacity was used. The experiment lasted for twelve weeks. Aeration was provided using air pumps, and water in bowls was siphoned every two days to avoid fouling.

2.2. Experimental Fish

The *Cyprinus carpio* (common carp) fingerlings were obtained from the Fish Farm of Bauchi State Agricultural Development Program (BSADP) along Dass Road Bauchi. Twenty fingerlings of mean weight 1.77±0.25g were acclimatized for two weeks. After the period of acclimatization, the fishes were randomly distributed into the plastic bowls.

2.3. Diet Formulation

Raw watermelon seeds were cleaned, sundried and milled to produce raw meal of watermelon seeds; they were then stored for diets formulation. The soybeans used were toasted for 30 minutes in an electric oven set at 100˚C and milled after cooling to get a soybean meal (SBM). The yellow maize used was milled also to get a yellow maize meal (YMM); the selected feed ingredients were stored in an air-tight and moisture-free container. Analysis was carried out to determine the nutrient composition of the raw watermelon seed meal diets, as well as the experimental fish before and after the experiments.

The experimental diets were formulated using Pearson square method to contain the same level of crude protein at 35% to meet the protein requirement of *Cyprinus carpio* fingerlings as stated by Hossain *et al*. (1997).

As a result, five experimental diets were formulated with raw watermelon seeds meal at different inclusion levels: Diet 1 (DT1; 0%), Diet 2 (DT_2; 5%), Diet 3 (DT3; 10%), Diet 4 (DT4; 15%) and Diet 5 (DT_5; 20%) while Coppens® fish feed Diet 6 (DT_6) was included as reference diet among the experimental diets.

2.4. Experimental Design and Management

The dietary treatments were in triplicates using Completely Randomized Design (CRD). After the acclimation period, fish were weighed and randomly distributed in bowls, each replicate contained 20 fingerlings of *C. carpio*. The experimental fish were fed twice daily (9.00 AM and 4.00 PM) at 5% body weight for twelve weeks.

The fingerlings were weighed every week to determine weight gain, and feed quantity given was adjusted accordingly. The growth parameters determined include mean weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio, apparent net protein utilization and survival rate.

2.5. Computation of the Growth Parameters

Calculation of the growth parameters were done according to the formulae described by Balfour (1998).

2.5.1. *Mean Initial Weight:* Twenty **(**20) of *C. carpio* fingerlings were counted and weighed, the total weight obtained was divided by 20, to obtain the mean initial weight of the fingerling.

MIW= N_W/N Where MIW =Mean Initial Weight N =Number of fingerlings W = Weight of fingerling

2.5.2. *Mean Final Weight:* The surviving fingerlings were counted and weighed; the weight obtained was divided by the number of the surviving fingerlings to obtain the mean final weight:

$$MFW = N_{sf}W/N_{sf}$$

Where MFW =Mean Final Weight (g)
N_{sf} =No. of surviving fish
W = Weight of the fish

Mean Weight Gained **=** Mean Final Weight – Mean Initial Weight

2.5.3. Survival Rate:

$$\text{Survival (\%)} = \frac{No - Ne}{No} \times 100$$

No = Initial total number of fingerlings
Ne = Total number of fish mortality at the end of feeding trial (12 weeks)

2.5.4. *Specific Growth Rate (SGR):* This parameter was determined according to the formula stated below:

$$SGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \times 100$$

Where ln = natural logarithm
W_2 = final weight
W_1 = initial weight
T_2 - t_1 = time duration (in days)

2.5.5. *Feed Conversion Ratio (FCR):* According to the formula stated below:

$$\mathbf{FCR} = \frac{Feed\ Intake}{Weight\ Gain}$$

2.5.6. *Protein Efficiency Ratio (PER):* It was determined according to the formula stated below:

$$\mathbf{PER} = \frac{Weight\ Gain}{Protein\ IntCake}$$

Where protein Intake = $\frac{\%\ Protein\ in\ Diet \times Total\ Feed\ Consumed}{100}$

2.5.7. *Apparent Net Protein Utilization (ANPU):* According to the formula stated below:

$$\mathbf{ANPU} = \frac{Protein\ Gained}{Protein\ Consumed} \times 100$$

Where protein gain = $Final\ Carcass\ Protein - Initial\ Carcass\ Protein$

2.6. Proximate Analysis of the Experimental Diets

The proximate composition of differently processed watermelon seed meal diets were carried out at Grand Cereals Jos. Moisture content, protein, ether extract, crude fiber, ash and nitrogen free extract of the diets were determined before the experiment, and those of the carcass of the experimental fish were determined before and after the feeding trial according AOAC (2000).

2.7. Data Analysis

Data obtained from the feeding trials were subjected to analysis of variance (ANOVA) where significant differences were observed between treatments; the means were compared using Fishers Least Significant Difference of the means (LSD). Genstat Discovery Edition 4 and Minitab 14 software were used for statistical analysis.

3. Results

The proximate composition of the experimental diets containing varying levels of raw watermelon seed meal diets is presented in Table 1. The diets were isonitrogenous at approximately 35% crude protein. DT_5 (20% inclusion) had the highest value for ether extract of 12.31±0.10% among the formulated diet, whereas DT_1 (0% inclusion) contained the least value of 8.53± 0.11%; however, the reference diet had a higher value compared to all formulated diets. The highest crude fiber was observed in diet 20% inclusion ($DT_{5,}$ 11.81±0.12%); whereas the reference diet (DT_6) had the least value (2.01±0.01%). DT_3 (10% inclusion) had the lowest ash content of 10.26±0.02%, while DT_6 (reference diet) had the highest values of 14.80±0.10%. The moisture content ranged between 10.76±0.02% to 11.89±0.11%, the highest value was recorded in DT_2 (5% inclusion) whereas the nitrogen free extract (NFE) was highest in DT_1 (28.89±0.33%) and lowest in DT_6 (3.43±0.08%).

The growth parameters, nutrient utilization and survival rate of common carp fingerlings fed varying levels of raw watermelon seed meal diets are presented in Table 2. The Mean Final Weight (MFW), Mean Weight Gain (MWG), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE), Protein Efficiency Ratio (PER), Apparent Net Protein Utilization (ANPU), Specific Growth Rate (SGR) and Survival Rate (SR) of fish fed all the experimental diets differed significantly (P<0.05).

The mean final weight of the fish fed DT_3 (10% inclusion level of watermelon seed meal) was significantly high (15.33±0.04g), while DT_5 had the lowest value of 11.58±0.20g. The Mean Initial Weight (MIW) of the experimental fish was 1.775± 0.00, showing no significant difference (P>0.05). The mean weight gain (MWG), feed conversion ratio (FCR) and feed conversion efficiency (FCE) differed significantly (P<0.05) and were best with fish fed DT_3 with values of 13.55±0.04g, 1.75±0.00 and 57.15±0.02, respectively. DT_5 had the least MWG (9.81±0.02g) and the least FCE (48.26+0.12) while DT_3 and DT_6 showed the best FCR of 1.75. The protein efficiency ratio (PER), apparent net protein utilization (ANPU) and specific growth rate (SGR) also differed significantly (P<0.05) with the highest values of 1.65±0.00, 23.73±0.11 and 2.57±0.00, respectively, recorded from the fish fed DT_3, while the lowest values 1.38±0.01, 18.03±0.09 and 2.23±0.00, respectively, were obtained from the group of fish fed DT_5. The percentage survival ranged from 93.30± 3.33% (DT_5) to 100±0.00% in $DT_{2,}$ DT_3 and DT_4, respectively.

Table 1. Proximate Composition of Diets containing different inclusion levels of raw watermelon seed meal

Treatment	Moisture %	Crude Protein %	Ether Extract %	Crude Fiber %	Ash %	NFE %
Diet 1 (DT_1)	10.76±0.02^c	35.23±0.06^b	8.53±0.11^d	5.70±0.08^e	10.89±0.02^b	28.89±0.33^a
Diet 2 (DT_2)	11.89±0.11^a	35.26±0.08^b	11.61±0.07^c	7.26±0.08^d	11.23±0.15^b	22.75±0.14^c
Diet 3 (DT_3)	10.77±0.01^c	34.70±0.05^c	12.26±0.10^b	8.54±0.11^c	10.26±0.02^c	23.47±0.07^b
Diet 4 (DT_4)	11.50±0.12^b	34.83±0.14^c	12.28±0.11^b	10.15±0.10^b	11.12±0.07^b	20.12±0.09^d
Diet 5 (DT_5)	11.00±0.12^c	34.99±0.11^{bc}	12.31±0.10^b	11.81±0.12^a	11.16±0.14^b	18.73±0.09^e
Diet 6 (DT_6)	10.97±0.08^c	52.24±0.15^a	16.55±0.08^a	2.01±0.01^f	14.80±0.10^a	3.43±0.08^f

Means in the same column followed by different superscripts letters differed significantly (P<0.05)

Table 2. Growth Parameters of the *Cyprinus carpio* Fingerlings Fed different inclusion levels of raw watermelon seed meal Diets

Treatment	MIW	MFW	MWG	FCR	FCE	PER	ANPU	SGR	% Survival
Diet 1	1.775±0.00	12.45±0.03^e	10.68±0.03^e	1.98±0.01^b	50.45±0.16^d	1.43±0.01^d	19.46±0.18^c	2.32±0.00^e	96.67±1.67^{ab}
Diet 2	1.775±0.00	14.99±0.05^c	13.21±0.05^c	1.81±0.01^d	55.29±0.25^b	1.57±0.01^b	21.56±0.15^b	2.54±0.00^c	100.00±0.00^a
Diet 3	1.775±0.00	15.33±0.04^b	13.55±0.04^b	1.75±0.00^e	57.15±0.02^a	1.65±0.00^a	23.73±0.11^a	2.57±0.00^b	100.00±0.00^a
Diet 4	1.775±0.00	13.47±0.05^d	11.69±0.05^d	1.90±0.01^c	52.60±0.23^c	1.51±0.01^c	21.45±0.20^b	2.41±0.00^d	100.00±0.00^a
Diet 5	1.775±0.00	11.58±0.02^f	9.81±0.02^f	2.07±0.01^a	48.26±0.12^e	1.38±0.01^e	18.03±0.09^d	2.23±0.00^f	93.30±3.33^b
Diet 6	1.775±0.00	16.28±0.04^a	14.50±0.04^a	1.75±0.01^e	57.05±0.27^a	1.09±0.01^f	16.31±0.09^e	2.64±0.00^a	100.00±0.00^a

Means in the same column followed by different superscripts letters differed significantly (P<0.05)
MIW: Mean Initial Weight; MFW: Mean Final Weight; MWG: Mean Weight Gain; FCR: Feed Conversion Ratio; FCE: Feed Conversion Efficiency; PER: Protein Efficiency Ratio; ANPU: Apparent Net Protein Utilization; SGR: Specific Growth Rate

Figure 1 shows the trend of weekly weight gain of common carp (*Cyprinus carpio*) fingerlings fed different inclusion levels of raw watermelon seed meal diets for the 12 weeks experimental period. The results showed significant different (P<0.05) among the treatments. The increase in weight gains was recorded for all the fishes fed the experimental diets. The highest weight gain was observed for fish fed diet DT6 (16.28g) followed by DT_3 (15.33g) while the lowest weight gain was recorded by fish fed DT_5 (11.58g) at the end of the feeding trial.

The initial and final proximate composition of the carcasses of the fish fed the experimental diets containing various levels of raw watermelon seed meals are presented in Table 3. The proximate analysis revealed significant differences (P<0.05) for moisture content, crude protein, ether extract, crude fiber, ash and Nitrogen Free Extract (NFE) of the experimental fish carcass before and after the feeding trial.

The result of proximate analysis of fish carcass fed the experimental diets indicated an increase in crude protein, ether extract and ash but a decrease in moisture content and crude fiber when compared with the initial carcass. The fish fed DT_5 had the highest carcass moisture content (79.58±0.03%), while the lowest value was observed in fish fed DT_6 (78.60±0.00%). The fish fed DT_6 had the highest carcass crude protein (14.59±0.01%) followed by DT_3 (14.10±0.06%), while DT_5 fish carcass had the lowest value (12.87±0.02%). Ether extract and crude fiber were highest in carcass of the fish fed DT_6 and DT_3 (4.02±0.01% and 0.10±0.01%, respectively), while DT_2 (2.75±0.06%) had the least ether extract and DT_6 fish carcass had the lowest crude fiber (0.04±0.01%). DT_5 had the highest ash content 3.32±0.03% and the lowest was recorded in the carcass of DT_6 (2.56±0.01%).

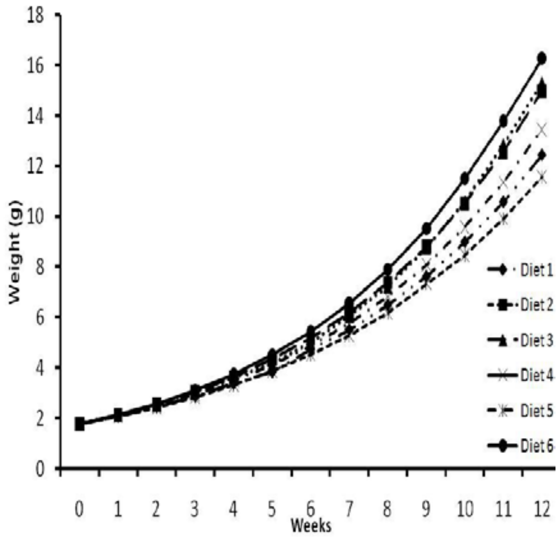

Figure 1. Weekly Weight Gain of *C. carpio* Fed different inclusion levels of raw watermelon seed meal Diets

Table 3. Proximate composition of carcass of fish fed different inclusion levels of raw watermelon seed meal diets

Treatment	Moisture %	Crude Protein %	Ether Extract %	Crude Fiber %	Ash %	NFE %
Initial	80.20±0.06^{a}	11.75±0.06^{g}	1.87±0.06^{d}	0.10±0.00^{a}	2.15±0.01^{e}	3.93±0.02^{a}
Diet 1	78.70±0.01^{de}	13.34±0.06^{e}	2.82±0.01^{c}	0.09±0.01^{ab}	3.11±0.06^{b}	1.94±0.03^{b}
Diet 2	78.68±0.08^{de}	13.51±0.06^{d}	2.75±0.06^{c}	0.09±0.02^{ab}	3.15±0.03^{b}	1.84±0.06^{c}
Diet 3	79.30±0.12^{c}	14.10±0.06^{b}	3.04±0.03^{b}	0.10±0.01^{a}	2.72±0.06^{c}	0.74±0.01^{f}
Diet 4	78.84±0.06^{d}	13.88±0.01^{c}	2.81±0.01^{c}	0.06±0.01^{cd}	3.27±0.04^{a}	1.14±0.03^{e}
Diet 5	79.58±0.03^{b}	12.87±0.02^{f}	2.83±0.02^{c}	0.07±0.00^{bc}	3.32±0.03^{a}	1.33±0.02^{d}
Diet 6	78.60±0.00^{e}	14.59±0.01^{a}	4.02±0.01^{a}	0.04±0.01^{d}	2.56±0.01^{d}	0.23±0.01^{g}

Means in the same column followed by different superscripts letters differed significantly (P<0.05)

4. Discussion

Davies and Gouveia (2010) reported that the nutrient requirement for growth, reproduction and normal physiological function of fish must be met, like other animals; but fish have much higher requirement for protein, so feed mixture with 25-45% of raw protein are mainly used. Furthermore, Mazid *et al.*, (1997) reported that the protein requirement for carp should be between 35-45% until the size of the fish reaches approximately 50-60g. However, Wilson and Halver (2005) suggested that herbivorous and omnivorous fish require a diet with 25-35% crude protein. According to the report of Akiyama (1999), usually fish growth will be directly proportional to the level, if the level within the range of approximately 20 to 40% crude protein. Similarly Noor *et al.* (2011) reported that the protein requirement of carp is 35%, which is in line with the diet formulated for this study.

The present study revealed that the lipid content of the experimental diet ranged from 8.53±0.11 to 12.31±0.10. The result conforms to the report of Andras *et al.* (2011) who reported that the lipid content of larvae and fingerling feed should be around 8 to 15%, the oil content would be increased until it reaches 12-15% according to the species and size of the fish. It was also observed, from the results, that the crude fiber contents in this study were higher than the values reported by Gatlin (2011) who indicated that cellulose and other fibrous carbohydrate were found in the structural component of plant and are indigestible to monogastric (simple-stomach) animals, including fish. In fact, the amount of crude fiber in fish feed is usually suggested to be less than 7% of the diet to limit the amount of undigested materials entering the culture system.

The growth parameter in this study showed that the mean final weight (MFW) and mean weight gain (MWG) of fish fed experimental diets differed significantly (P<0.05). Both the MFW and MWG of DT_2, DT_3 and DT4 were better than DT_1 and DT_5. However, fish fed (DT_3) 10% level of watermelon meal was significantly (P<0.05) higher compared to the fish fed control DT_1 (0% inclusion) and the remaining diets. This is similar to the result of Shazali *et al.* (2013) who reported that the inclusion of watermelon seed meal for broiler diets up to 10% significantly induced better growth and feed utilization efficiency.

This may be attributed to differences in the level of ether extract, fiber content and carbohydrate (NFE) of the experimental diets. This result conforms to the report of Craig and Helfrich (2002) that lipid fats are high-energy nutrients that can be utilized to partially spare protein in aquaculture feed and typically comprise about 10 to 25% of the fish diet. Moreover, the result collaborates with the findings of Oladunjoye *et al.* (2005) that the high fiber content could be responsible for growth depression. Similarly, Lovel and Leary (1990) pointed out that increasing fiber content beyond the basal level could reduce the growth of fish due to poor digestion of cellulose. This is likely to be responsible for the poor growth performance of fish fed DT_5 (20% inclusion) containing a high crude fiber. This also agrees with the findings of Sawaya *et al*. (1986) who recommended that the watermelon seed should not be included at levels higher than 20%, because these levels bring up the fiber content of the ration to be over 10%, which reduces the feed intake.

The results of this study also reveal that the feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER), apparent net protein utilization (ANPU) and specific growth (SGR) followed the same trend with mean weight gain (MWG) which revealed that DT_3 and DT_2 had the best growth performance. However, all the experimental fish exhibited good SGR values (>2% body weight) using the standards reported by FAO (2004) that daily growth rate of carp can be between 2 to 4% body weight. This might be influenced by the variation in crude fiber, fat content and NFE in the diets. This result agrees with the report of Craig and Helfrich (2002) who pointed out that protein is used for growth if adequate levels of fat and carbohydrates are present in the diet; if not, protein may be used for energy and life support rather than growth. Increasing the dietary lipid with high quality fats improves growth, feed conversion and protein utilization, thus reducing nitrogen excretion (Steffens, 1993).

The study also shows a higher percentage survival rate (93.30±3.33-100±0.00%) of the common carp fingerlings fed the experimental diets during the 12 weeks experimental period. This shows that all diets formulated were not harmful to the fish and agrees with the findings of Basavarajah and Anthony (1997) who reported a survival rate of 98% for common carp fry fed conventional feed and 100% for fry fed supplementary feed for a 35-day feeding trial. Similarly, Singh and Dhawan (1996) pointed out that 100% survival rate of carp can be achieved under a very minimal stress and a well-fed condition.

The proximate composition of the experimental fish carcass revealed that the crude protein and lipid content in the fish fed DT_3 is higher than the initial carcass and the fish fed remaining diets. Fish fed DT_2 and DT_4 had a moderate crude protein content in their carcass; however, the results exhibited that all the fingerlings utilized the diets well. The difference may be a result of high lipids and low crude fiber content in DT_3 compared with the rest of the diets containing watermelon seed meal. This is in agreement with the findings of Abbas (2007) and Manjappa *et al.* (2011) who pointed out that a better utilization of nutrients in the carcass of fish fed high lipids diets and protein utilization for growth are related to both the dietary protein level and the availability of non-protein energy sources with lower inclusion of dietary fiber.

5. Conclusion

The feeding trial on varying inclusion levels of raw watermelon seed meal revealed that DT_3 which had 10% inclusion level emerged as the best in both growth performance and nutrient utilization, while DT_5 (20% inclusion level) was the least and poorest growth performance among the treatments. Inclusion level of 10% raw watermelon seed meal in the diet of common carp is suggested to enhance growth performance and nutrient utilization.

As evidenced by the findings of this study, raw watermelon seeds can be successfully used in common carp feed at 10% without adverse effects on the growth responses and nutrient utilization of the fish; further studies should be performed on processing raw seeds so as to improve their growth potential.

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