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THE NUTRITIONAL VALUE OF COW BLOOD MEAL AND ITS EFFECTS ON GROWTH PERFORMANCE, HAEMATOLOGY AND PLASMA ENZYMES OF HYBRID CATFISH

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Target Audience: Researchers in aquaculture, fish farmers, feed millers.

ABSTRACT

The nutritional potential of sun-dried cow blood meal (CBM) was investigated for its effect on growth performance, haematology and plasma enzyme activities in hybrid catfish fingerlings (*Clarias gariepinus Male* x *Heterobranchus longifilis* Female), in an assay that lasted for twelve weeks. Six graded diets containing 0, 5, 10, 15, 20 and 25 percent of CBM were compounded. Ten fingerlings (mean weight 4.23±0.64g) per tank were reared under laboratory conditions in eighteen plastic tanks, each containing 12 litres of water. The fingerlings were fed 3% of their body weight daily, with bimonthly adjustments. At the end of the 84-day experiment, growth performance parameters such as weight gains, specific growth rate (SGR) and feed conversion ratio (FCR) were determined. Blood samples were pooled from each treatment on days 0, 42 and 84 for haematological and plasma enzyme analyses.

The specific growth rate and the feed conversion ratio were best in the fish fed the control (0% sun-dried CBM) diet and poorest in the fish fed 25% CBM diet inclusion. There were no significant differences (P>0.05) between mean daily weight gains, specific growth rate and feed conversion ratios of fish fed diet 2 (5% CBM) and the control diet. The higher the inclusion level of the sun-dried CBM in the experimental diets, the poorer the nutrient utilization and growth performance. Haematological and plasma enzyme analyses revealed progressively severe macrocytic normochromic anaemia, leucocytosis and high enzymatic activities of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) in fish fed increasing levels of sun-dried CBM. These enzymatic changes imply liver, kidney and/or cardiac damage, and opportunistic systemic bacterial infection. It can be inferred that for optimum growth performance, best nutrient utilization and unimpaired haematological and plasma enzyme activities in hybrid *Clarias*, a 5% level of sun-dried CBM inclusion in their diet is recommended.

Key words: Nutritional value, Cow blood meal, Growth performance, Haematology, Plasma enzymes, Hybrid catfish.

DESCRIPTION OF THE PROBLEM

Aquaculture as a source of animal protein is becoming increasingly important, confirming the reported (1) estimate of about ten million people dying yearly of starvation or malnutrition in the world. The dwindling availability of fish and fish products coupled with the increasing catching pressures on marine fish resources have highlighted the vital role of fish farming in meeting the world's future food requirement. It was reported (2) that the world's population has doubled to 6.1 billion in the last 50 years and is expected to increase further by 3.3 million over the next 50 years. This level of growth will place enormous new demands on global food supplies. Therefore, farmed fish is expected to be very much in the frontline of any future struggle to feed the world, since the oceanic fish catch is no longer increasing. It can be inferred that the two million tonnes annual growth in protein supply, which used to come from captured fishes, will in future be provided by farmed fish, poultry and other protein producing sources (2).

Despite the role of aquaculture in world fish production, Africa still lags behind the world in global pisciculture (1, 2). Much research is however going on in expanding the aquacultural sector in the continent. Much effort has been put in areas of nutrient requirements, biology, ecology, reproduction and growth of some cultivable local species in the continent (3). Fish farming offers some advantages over the farming of domestic land animals (4). One such advantage is the ability of many species of fish to convert organic waste such as sewage, piggery wastes, poultry wastes, cow dung and other organic industrial by-products into useful protein efficiently, thus contributing to the management of waste in our environment (5). Table fish reared under aquacultural conditions are often subjected to environmental changes or stress such as water quality deterioration, competition for food between individual fish, handling, changes in ambient temperature and crowding (6). Since fish blood is sensitive to a variety of environmental changes it is possible that certain characteristic of fish blood may register feeding effects (7). However, feeding is recognized as one of the important factors affecting growth rates (8). Many methods through the study of blood, respiration, hormone metabolism, digestion and osmoregulation are useful for the investigation of fish health. Detailed comparative studies on the health of man and livestock show their blood as a unique medium in which all processes taking place in animals are reflected (9). Haematology is important in assessing fish health and biological processes in different species of fish, while plasma biochemistry helps in the understanding of the changes due to tissue, hormonal and homeostatic variations which affect changes in levels of minerals, protein enzymes, lipids and vitamins in the blood (10). Hence, there is a need to study the plasma biochemistry of the test fish. The study is therefore designed to evaluate the nutritional potential of various concentrations of cow blood meal

(CBM) inclusion on the growth, haematological and plasma biochemistry of hybrid catfish fingerlings.

MATERIALS AND METHODS

Fresh cow blood was purchased from a local abattoir in Ibadan, Nigeria, boiled for one hour, sun-dried for 3 days and blended into fine powder. The other ingredients were obtained from a livestock feed store in Ibadan, Nigeria. Six diets – 1, 2, 3, 4, 5 and 6 were formulated with varying proportions - 0%, 5%, 10%, 15%, 20% and 25%, respectively of sun-dried cow blood meal (CBM) included into the basal diets. The control diet had 0% CBM inclusion. The crude protein level of the diets was 40% (11). The experimental diets were weighed, thoroughly mixed, moistened, pelleted, dried for 4 hours and stored at -18°C in polythene bags until used. Ten fingerlings of hybrid catfish, mean weight 4.23±0.64g, were allotted per tank with three replicates per treatment. Fresh water was supplied from connected tap at 0.25l/min to replace water loss by evaporation. The water temperature, dissolved oxygen, pH, ammonia, nitrate and nitrite levels were monitored weekly. The fingerlings were fed 3% of their total body weight daily at 10.00hr after and 18.00hr and were weighed bimonthly. The quantity of feed fed to the fish was adjusted relative to the weight gained. The diets and carcasses were analyzed for moisture, protein, fat, fibre and ash content using standard analytical methods (12). Blood was pooled from randomly selected samples from each treatment both at the initial (day 0), middle (day 42) and at end of experiment (day 84) for haematological, and plasma enzymes - alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) analyses (13). Data obtained were analysed using the one-way analysis of variance and comparison of means (14).

RESULTS AND DISCUSSION

The water quality parameters for all the experimental tanks were within acceptable ranges as recommended (15) for fish culture.

Proximate analyses of the diets

The proximate composition of sun-dried CBM is as follows: crude protein 81%, Ash 2.3%, crude fibre 1.5%; moisture 4.05, fat 0.60%; NFE 10.55%. The results of the proximate analysis of the sun-dried CBM, the gross and proximate analysis of the experimental diets are recorded in Tables 1.0(a) and (b). The proximate analysis results showed that the experimental diets contained an average of 40.66% crude protein and they were well accepted by the test fish. Reseachers (11, 16) have reported that the African catfish in its early stage requires 36–41% crude protein for growth. However, the protein requirement declines to about 30% after the fingerling stage.

Table 1.0(a): Gross Composition of Experimental Diets

	Diets					
Ingredients	1	2	3	4	5	6
Fishmeal	52.1	47.1	42.1	37.1	32.1	27.1
Cow blood meal	0.0	5.0	10.0	15.0	20.0	25.0
Yellow maize	22.3	22.3	22.3	22.3	22.3	22.3
Cassava flour	22.3	22.3	22.3	22.3	22.3	22.3
Vitamin premix	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix	0.0	0.6	0.6	0.6	0.6	0.6
Groundnut/Cod liver oil	2.0	2.0	2.0	2.0	2.0	2.0
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Total	100.0	100.0	100.0	100.0	100.0	100.0

Table 1.0(b): Proximate Composition of Experimental Diets

	Composition (% dry weight)					
Diets	Crude Protein	Ash	Crude fibre	Moisture	Lipids	NFE
1	40.80	10.6	1.4	13.9	3.1	30.2
2	40.6	9.3	1.7	13.7	0.8	34.0
3	41.1	8.7	2.3	14.0	2.3	31.7
4	40.5	8.4	2.2	13.5	2.7	32.7
5	41.0	8.9	2.6	14.6	2.6	30.6
6	40.1	10.6	2.6	13.1	2.3	31.4
Mean	40.7±1.5	9.4±90.7	2.1±0.3	13.8±0.9	2.3±0.4	32±1.3

a,b,c: Values along the same column with different superscripts differ significantly from their respective mean values ($P<0.05$).

Proximate Composition of Experimental Fish

The proximate composition of the experimental fish before and after the feeding trials is presented in Table 2.0. Fish fed diet 1 with 0% CBM had the highest body protein of 77.5%. Fish fed diets 3 had (75.8% CP) and Diet 2 (75.1% CP) are significantly different ($p<0.05$) from fish fed diet 1 but insignificantly different ($p>0.05$) from each other and fish on Diet 4 (74.9%). Fish fed treatment 6 had the lowest carcass protein (65.6%). Conversely, fish on diet 6 had the highest level of ash while treatment 1 had the lowest level of ash.

The crude protein in the initial fish was 67.9%. There was a significant improvement in the crude protein (CP) stored in the flesh of all the fish fed the experimental diets when compared with CP of the initial fish. Only treatment 6 had a lower CP than the initial (65.6%) at the end of the experiment. The

Table 2.0: Proximate composition of fish before and after experiment

	%Crude protein	%Ash	% Fibre	% Moisture	% Lipid
			Before Experiment		
	67.9	15.0	0.007	15.0	1.6
Diets			After Experiment		
1	77.5a	13.0c	0.0001c	9.0d	1.5
2	75.1b	13.0c	0.0008b	11.0c	1.3b
3	75.8b	13.5b	0.0010b	10.0c	1.2b
4	74.9b	14.0b	0.0003d	10.1c	0.9d
5	70.3c	15.0a	0.0006c	14.0b	1.1c
6	65.6d	15.1a	0.0050a	17.5a	1.0c
Mean	73.2±2.0	13.9±0.9	0.0008±0.00013	12.1±0.3	1.2±0.3

a,b,c: Values along the same column with different superscripts differ significantly from their respective mean values ($P<0.05$).

negative growth in treatment 6 could only mean that fish muscle protein was broken down and used for energy generation.

Fish fed diet 2 with 5% cow blood meal (CBM) inclusion level is significantly different ($p<0.05$) from diet 1 in the carcass protein fibre moisture and lipid composition but insignificantly different ($p>0.05$) from diets 3 and 4. The control and diet 2 are however, statistically similar in ash composition.

The best growth performance was achieved with fish fed diets containing 0% blood meal (40.8% CP) while the fish raised on feed containing the highest amount of CBM (25%) gave the poorest performance in terms of growth and protein efficiency ration (PER). Similar observations were reported for fish fed increasing levels of CBM (4, 16, 17, 18). The mean daily weight gain, final weight gain and percentage weight gain were higher in diet 1 followed by diet 2. The poorest values were obtained in diet 6. Those observations were probably due to the reduced palatability of the feed as levels of CBM increased in the diets.

The lipid levels in the diets were lower than those observed in a previous work (19) where diets containing 8% lipids leads to higher levels of protein deposition because they tend to spare protein for usage as energy sources. Six percent lipid level is recommended as ideal in fish growth (4). However, recent research studies demonstrated protein sparing at lipid levels up to 16% in African catfish diets (20).

The increased moisture retention in the carcass of the fish at higher levels of CBM in the diets is related to the decrease in the fat content within the fish muscles. Water in the fish is held at the expense of lipids. The inverse relationship between carcass moisture content and lipid has been reported (4, 21, 22).

Growth Performance and Nutrient Utilization

Table 3.0 shows the growth performance and nutrient utilization of the hybrid catfish fed the test diets for 84 days. The mean initial weight of test fish fed the control and diet 6 were significantly different ($p < 0.05$) from the other diets and from each other. Fish on the control diet had the best specific growth rate (SGR) of 1.78/day while fish on Diet 6 had the lowest SGR of 1.2g/day. Fish raised on treatment 2 had a higher and better growth (15.6g) than fish fed Diet 6 (15.2g), which had a mean initial higher weight of 5.5g as against 4.1g of fish fed diet 2. The specific growth rate (SGR) of fish fed the control, diet 2, and 4 are insignificantly different ($p > 0.05$). The fish fed diets 3 and 5 have exactly the same SGR. The feed conversion ratio (FCR) of the control diet was the best (2.4) while the FCR of fish fed diet 6 was the poorest 5.2. The FCR of all the test diet were significantly different ($p > 0.05$) from one another. However, the

Table 3.0: Growth performance and nutrient utilization of hybrid catfish fed varying inclusion levels of sun-dried CBM

Growth parameters	Diets 1	2	3	4	5	6	Grand mean
Mean initial weight (g)	3.5	4.1^b	4.3^b	3.9	4.1	5.5	4.2±0.5
Mean final weight (g)	16.9	15.6	14.8	14.1	14.4	15.2	15.1±0.9
Mean daily wt. gain (g/day)	0.16	0.14	0.13	0.12	0.12	0.12	0.13±0.08
Mean weekly wt. gain (g/wk)	1.12	0.96	0.88	0.85	0.85	0.81	0.91±0.21
Total percentage wt. gain (%)	385.00	277.70	247.06	254.25	247.6	171.7	262.39±3.60
Specific growth rate (g/day) (SGR)	1.78	1.58	1.48	1.54	1.48	1.20	1.51±0.39
Total feed intake (g)	31.5	30.5	35.5	30.3	37.56	49.98	35.89±1.41
Mean feed intake per fish (g)	0.81	0.62	0.84	0.71	0.80	1.09	.90±0.21
Feed conversion ratio (FCR)	2.4	2.7	3.4	3.0	3.7	5.2	3.36±0.43
Gross efficiency food conversion (GEFC)	42.5	37.6	29.6	33.7	27.2	19.37	31.61±1.33
Protein intake fish (g) (PI)	12.9	12.4	14.8	12.2	15.2	19.99	14.58±0.90
Daily protein intake (g/day)	0.15	0.15	0.18	0.15	0.18	0.27	0.17±0.10
Protein efficiency ratio (PER)	1.043	0.93	0.71	0.83	0.67	0.43	0.78±0.21

Means with the same superscripts along the rows are not significantly different from the grand mean at $P < 0.05$.

PER of diet 2 was not significantly different from the control diet. The protein efficiency ratio (PER) which is a converse of the FCR indicate that the control diet had the best PER followed by treatments 2 (5% CBM) and 4, respectively while the others had lower PER. The PER of diets 2 and 4 were not significantly different ($p > 0.05$) from each other.

The decreasing trends in fish growth with increasing dietary cow blood meal (CBM) might be due to the nature of its imbalanced amino acid profile (16). For good growth, the CBM needs to be properly processed.

Haematology

Table 4.0 shows the variations of the haematological parameters with increasing days of feeding and within various diets. There were progressive decreases in PCV, Hb concentration and RBC counts with increasing inclusion levels of sun-dried CBM from the 10% level onwards.

Table 4.0: Haematological parameters of hybrid catfish fingerlings fed varying levels of sun-dried cow blood meal inclusion

Haematological Parameters	Diets 1	2	3	4	5	6
PCV (%)						
Day 0	35.5±0.6	34.0±1.1^{a}	29.3±0.4^{a}	29.5±0.5^{a}	25.5±0.5	23.5±0.5^{d}
Day 42	34.6±0.4	34.2±0.9	30.5±0.3^{b}	28.9±0.3	25.9±0.3	24.2±0.3^{d}
Day 84	35.7±0.3	34.1±0.4	28.9±0.6^{d}	29.2±0.2^{a}	27.1±0.3	25.6±0.5
Hb. conc. (g/dl)						
Day 0	10.5±0.5	9.8±0.3^{a}	8.6±0.3	8.4±0.2	7.6±0.2	7.0±0.2^{d}
Day 42	9.9±0.5	10.1±0.5^{a}	9.1±0.2^{b}	9.0±0.3	8.3±0.3	8.1±0.1
Day 84	11.2±0.3	10.9±0.3^{a}	8.9±0.4^{b}	9.1±0.2	8.6±0.3	8.3±0.2
RBC ($x10^{6}/\mu l$)						
Day 0	3.4±0.1	3.3±0.3^{a}	2.7±0.1^{b}	2.7±0.1^{b}	2.3±0.1^{c}	2.2±0.1
Day 42	3.3±0.1	3.2±0.3^{a}	2.8±0.2^{b}	2.7±0.5	2.4±0.3	2.2±0.2
Day 84	3.5±0.5	3.3±0.6^{ab}	2.7±0.4^{b}	2.6±0.4	2.5±0.4^{b}	2.3±0.2
MCV (fl)						
Day 0	104.2±0.7^{b}	103.0±1.1^{b}	108.6±1.5^{c}	109.3±2.7	110.8±2.5^{c}	106.8±2.3^{a}
Day 42	104.6±0.4	105.9±1.0^{b}	108.9±0.9^{a}	107.0±1.2^{a}	107.9±1.6^{a}	110.0±2.0^{a}
Day 84	102.3±1.3	103.3±0.9^{bc}	107.2±1.1^{ab}	112.3±0.9	108.4±1.2^{a}	111.3±2.1^{a}
MCHC (g/dl)						
Day 0	29.6±1.1	28.8±1.5^{a}	29.4±1.1^{a}	28.4±1.1^{a}	29.8±1.1^{a}	29.8±1.1^{a}
Day 42	28.7±1.6	29.5±1.1^{a}	29.5±1.3^{a}	30.2±1.1^{a}	32.0±2.3^{a}	33.5±2.6^{a}
Day 84	31.1±1.1^{a}	31.2±1.2^{a}	30.7±1.5^{a}	30.9±1.8^{a}	31.7±1.9^{a}	32.4±2.2^{a}
TWBC $x10^{3}/\mu l$						
Day 0	4.55±0.12	4.16±0.02	4.62±0.52	4.30±0.20	6.44±0.50	8.62±1.8
Day 42	5.12±0.10	5.03±0.03	4.96±0.34^{d}	5.09±0.33	5.96±0.23	6.23±1.0
Day 84	4.23±0.22^{d}	4.52±0.06^{d}	4.60±0.22^{d}	4.81±0.22^{d}	5.23±0.21^{a}	5.53±0.9^{a}

a,b,c,d: Means within the same row and different days with different superscripts differ significantly ($p<0.05$).

These changes became more severe with increasing days of feeding. However, no significant variations in haematological parameters occurred between diets 1 (0%) and 2 (5%) level of sun-dried CBM.

These results show progressive macrocytic normochromic anaemia, with increase in CBM inclusion in the diets and increasing days of consumption. This is an indication of improved bone marrow erythropoietic response to an ensuing anaemia resulting from low feed intake and possible metabolic dysfunction in organs like the liver and kidney. The liver and kidneys are responsible for the synthesis of erythropoietin, an important hormone in bone marrow erythropoiesis (23).

Increased total white blood cell counts (TWBC; leucocytosis) was observed in hybrid *Clarias* fed 20 and 25% sun-dried CBM inclusions (diets 5 and 6, respectively; Table 4.0). This is an indication of increased production or egress of leucocytes into peripheral circulation from the marginated pool, the bone marrow or spleen, probably in response to bacterial infection in the already compromised and anaemic fish (13).

Plasma Enzymes Analysis

Results of the plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) analysis among the various diets are recorded in Table 5.0. There were significant increases ($p<0.05$) in the levels of all the enzymes analyzed, especially AST and GGT in diets 3, 4, 5 and 6 and with increasing days of feeding. The increases recorded in all the enzymes indicate clinical signs of tissue pathology, especially of the liver, heart and kidneys (24, 25). These changes in enzyme levels follow the same pattern of anaemia and leucocytosis, suggesting occult organ damage (13, 26) and systemic infection, probably with opportunistic bacteria either in the body or in the water of the aquaria.

Table 5.0: Plasma enzymes levels of hybrid catfish fingerlings fed varying levels of sun-dried cow blood meal inclusion

Enzymes	Diets					
(I.U/ml)	1	2	3	4	5	6
ALT						
Day 0	35.8 ± 2.5^{c}	33.6 ± 2.9^{e}	34.6 ± 2.3^{c}	39.7 ± 1.2^{b}	61.4 ± 2.6^{a}	65.2 ± 0.5^{a}
Day 42	36.1 ± 2.2^{b}	38.2 ± 2.1^{c}	39.2 ± 1.2^{b}	41.1 ± 2.2^{b}	66.1 ± 1.9^{a}	66.2 ± 2.0^{a}
Day 84	34.8 ± 1.9^{c}	33.9 ± 2.3^{c}	34.1 ± 5.5^{c}	42.5 ± 0.9^{b}	64.7 ± 1.3^{a}	68.8 ± 0.9^{a}
AST						
Day 0	23.0 ± 1.1^{d}	23.3 ± 0.9^{d}	29.9 ± 2.1^{c}	33.3 ± 1.4^{b}	43.0 ± 0.2^{a}	45.2 ± 1.6^{a}
Day 42	24.1 ± 2.3^{d}	25.2 ± 2.6^{d}	28.2 ± 2.4^{c}	34.2 ± 0.3^{b}	44.0 ± 2.5^{a}	51.1 ± 1.3^{a}
Day 84	23.3 ± 1.6^{d}	24.2 ± 0.9^{d}	29.6 ± 1.3^{c}	36.5 ± 1.7^{b}	52.3 ± 2.4^{a}	55.6 ± 1.7^{a}
GGT						
Day 0	11.3 ± 0.5^{e}	12.1 ± 0.2^{e}	14.4 ± 0.7^{d}	17.1 ± 1.5^{c}	21.8 ± 1.6^{b}	23.3 ± 0.9^{a}
Day 42	10.9 ± 0.3^{e}	12.2 ± 0.5^{d}	16.8 ± 0.5^{c}	16.9 ± 0.5^{c}	22.6 ± 2.3^{b}	25.1 ± 0.6^{a}
Day 84	9.9 ± 0.6^{e}	10.9 ± 0.2^{e}	15.1 ± 1.1^{d}	18.5 ± 1.9^{c}	20.3 ± 1.7^{b}	21.3 ± 0.2^{b}
ALP						
Day 0	137.0 ± 2.7^{d}	139.0 ± 1.5^{d}	147.0 ± 2.7^{c}	157.1 ± 2.9^{b}	159.3 ± 1.9^{b}	166.1 ± 1.8^{a}
Day 42	141.2 ± 3.5^{d}	140.1 ± 3.3^{d}	148.1 ± 2.2^{c}	156.1 ± 1.6^{b}	161.2 ± 2.1^{b}	171.3 ± 2.2^{b}
Day 84	136.5 ± 3.3^{e}	137.6 ± 2.1^{e}	146.6 ± 1.4^{d}	151.2 ± 1.3^{c}	166.4 ± 1.3^{b}	169.9 ± 1.4^{a}

a,b,c,d,e: Means within the same row and different days with different superscripts differ significantly ($P<0.05$).

CONCLUSION AND APPLICATIONS

(1) The study revealed that the best growth performance after the control diet was achieved with the diet containing 5% CBM and the poorest growth performance was obtained with diets containing 25% CBM. It can be derived that increased level of CBM in the diets of livestock and fish make the diets unpalatable and hence cause under-nutrition or malnutrition and poor growth performance.

(2) Haematological and plasma enzyme analyses revealed a macrocytic normochromic anaemia, leucocytosis and high plasma enzyme activities with increasing levels of CBM inclusion and time of consumption. This might have been caused by under-nutrition or malnutrition and consequent derangement in organ and tissue metabolism, damage and dysfunction, and susceptibility to opportunistic infections.

(3) CBM is extremely high in protein (about 81%) but low in isoleucine (27). The high lysine content in CBM if mixed with corn bran, which is low in lysine but high in isoleucine can be compounded to make balanced fish diets. The animal protein source (CBM) is often obtained at little or no cost from Nigerian local abattoirs where it is largely discarded. A researcher (18) observed that CBM is not very palatable to most livestock and hence not very popular as a protein supplement in livestock feeds.

(4) CBM has a great potential as an inexpensive effective and readily available protein supplement in aquaculture diets.

(5) It can be concluded that sun-dried cow blood meal can best be included in the diets of catfish fingerlings at 5% replacement level. At this level it can support fish growth with SGR, FCR and daily protein intake (g/day) as good as the fish fed fishmeal based diets containing an average of 40.66g/100g CP of the diet.

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