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Nutrient utilization of *Clarias gariepinus* juveniles fed diets of thermally processed toad meal as replacement for fishmeal

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ABSTRACT

Increasing cost of conventional feedstuffs has made it imperative to investigate into the utilization of the unconventional ones. The utilization of thermally processed toad meal as a replacement for fish meal was investigated. The toads were collected, processed and oven-dried at 1000C to a constant weight. The thermally processed toad meal was thereafter used to replace fish meal at replacement levels of 0%, 25%, 50%, 75% and 100% in diets 1, 2, 3, 4 and 5 respectively. Mean weight gain (MWG) differed significantly (p<0.05), with highest value recorded in diet 1 (20.83g ± 0.39) and lowest value in diet 3 (11.81g ± 0.78). Specific growth rate (SGR) differed significantly (p<0.05), ranging from 1.49%day-1 ± 0.07 in diet 3 to 2.14%day-1 ± 0.02 in diet 1. Thermally processed toad meal diets gave less growth performance indices than fishmeal, but could be a fair enough replacement for fish meal at 100% replacement level. Replacement of fishmeal with thermally processed toad meal indicated that their synergic effect was less than their individual effects.

INTRODUCTION

Fish feed is a major input in aquaculture production and a fundamental challenge facing the growth of aquaculture (Gabriel et al, 2007). This is aggravated by the high cost and scarcity of feed ingredients used conventionally. In recent times, the cost of conventional fish feed ingredients have increased quite rapidly due to their competitive use for livestock production and/or human consumption, thereby increasing the cost of fish production.

One of such conventional feedstuff is fishmeal which ranks as the most preferable protein source in fish feed manufacture. However, its supply is not adequate to sustain the growth of aquaculture in addition to the demand from other animal feed industries (Mondal et al., 2008). Thus, the increasing need to investigate and utilize more of the locally available unconventional feedstuffs to replace fishmeal in fish feed formulation.

Toad meal processed from toads of the Bufonid family has been reported to have good prospects as an unconventional protein feedstuff (Bekibele et al., 1995). It has been reported to have high protein content and comparable amino acid profile (Jimmy et. al., 2015). Despites these advantages, the presence of the poison gland in toads render toad meal unutilizable by feed industries.

However, deactivation of toxins and anti-nutrients in feedstuffs has been achieved using several processing methods. One of such method is heat processing (Isikwenu and Bratte, 1999; Tiamiyu et al., 2007). It is therefore imperative to attempt deactivating toxin in toadmeal by adopting thermal processing in order to render it fit for incorporation into feeds.

The African catfish, *Clarias gariepinu*s is reported to be the most important fish species cultured in Nigeria; it grows rapidly, it is disease and stress resistant, sturdy and highly productive in polyculture with many other fish species (Hammed, et al., 2015).

Therefore this work investigates the nutrient utilization of thermally processed toadmeal by *Clarias gariepinus*.

MATERIALS AND METHODS

Experimental Fish

Clarias gariepinus juveniles (mean weight 9.13 ± 0.02g) were purchased, acclimatized for 2 weeks and stocked in plastic bowls at 20 fish per bowl at the indoor hatchery of the Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Benue state, Nigeria.

Procurements and Processing of Feed Ingredients

The feed ingredients such as fishmeal, vitamins and mineral premixes were purchased from a reputable livestock feed shop in Makurdi. Soybean was purchased, toasted at 100^{o}C in an oven for 30minutes, allowed to cool and ground into a meal. Maize was purchased and ground into a meal.

With the aid of hand gloves and other protective apparatus, toads of the Bufonid family were harvested from streams along international market road, Makurdi. The toads were killed using club to knock them on the head to unconsciousness, followed by pithing as described by AVMA (2013), before they were gutted, washed and oven dried at 100^{o}C to a constant weight.

Diet Formulation

The diets were formulated using pearson square method with 35% crude protein to meet the requirement for C. *gariepinus* juveniles. The biochemical composition of thermally processed toad meal used for this formulation was as reported by Jimmy, et al., (2015). Thermally processed toad meal was used to replace fish meal at various replacement levels of 0%, 25%, 50%, 75% and 100% as diets 1,2,3,4 and 5 respectively. The control experiment was 0% dietary treatment (diet 1). The diets were composed of ingredients as shown in Table 1. The ingredients were ground, mixed, pelleted, dried into individual diets and stored at 40C in a fridge until required for use.

Experimental Set up and Management

Five (5) bowls were stocked randomly with twenty juveniles per bowl for this experiment. The fish were fed twice daily (morning and evening) with their respective diets at 5% fish body weight. They were weighed weekly to determine weight gain and the quantity of feed was adjusted accordingly. The feeding trial lasted for eight (8) weeks.

Experimental Design

The completely randomized design was used as the experimental design. The dietary treatments were assigned to groups at random in a completely randomized design and each treatment was in triplicate.

Analyses of Proximate Compositions

The proximate composition analyses of the experimental diets and samples of fish carcasses fed experimental diets at the start of the feeding trial and at the end of the experiment were carried out using AOAC (2005) standard method.

Determination of Water Quality

Water quality parameters such as temperature, total dissolved solids, electrical conductivity and pH were determined using Hanna waterproof tester H198129 and dissolved oxygen (Do) was measured using Lutron Do meter Do5509.

Measurement of Growth Parameters

Growth parameters measured such as weight gain, percentage weight gain, specific growth rate, food conversion ratio, protein efficiency ratio, apparent net protein utilization and survival rate were calculated as follows:

$$\text{Mean Weight Gain (MWG)} = \text{Mean Final Weight (MFW)} - \text{Mean Initial Weight (MIW)}$$

$$\text{Specific Growth Rate (SGR)} = \frac{\ln \text{MFW} - \ln \text{MIW}}{\text{T}} \times 100$$

Where ln=natural logarithm
MFW= mean final weight
MIW = mean initial weight
T = period of experiment in days

$$\text{Food Conversion Ratio (FCR)} = \frac{\text{weight of feed consumed (dry) in grams}}{\text{weight gain of fish produced (wet) in grams}}$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{mean weight gain of fish produced (wet) in grams}}{\text{weight of protein in feed (dry) in grams}}$$

Where: Mean weight gain = mean final weight – mean initial weight

$$\text{weight of protein} = \frac{\text{\% protein in diet} \times \text{total feed consumed}}{100}$$

$$\text{Apparent Net Protein Utilization (ANPU)} = \frac{\text{protein gained}}{\text{protein consumed}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{number of fish that survived}}{\text{number of fish stocked}} \times 100$$

Data Analysis of the Various Experiments

Data obtained were subjected to a one-way analysis of variance (ANOVA) where the means were separated using Least Significant Differences (LSD) at 95% confidence level (P < 0.05) using Genstat package edition 12.

RESULTS AND DISCUSSION

Table 2 shows the proximate composition of experimental diets of thermally processed toad meal as replacement for fishmeal. The result reveals that crude protein content of the experimental diets of thermally processed toad meal as replacement for fishmeal did not differ significantly (p>0.05), ranging from 35.35 ± 0.35 in diet 2 to 36.22 ± 0.25 in diet 3. This indicates that the differences observed in the results of the experiment were not as a result of bias in the level of protein content in the different diets.

Table 3 reveals the growth performance of Clarias gariepinus juveniles fed diets of thermally processed toad meal as replacement for fishmeal. The result shows that the mean initial weight (MIW) of Clarias gariepinus juveniles fed the different diets did not differ significantly (p>0.05). Mean final weight (MFW) differed significantly (p<0.05), ranging from 20.88g ± 0.77 in diet 3 to 29.85g ± 0.42 in diet 1. Mean weight gain (MWG) differed significantly (p<0.05), with highest value recorded in diet 1 (20.83g ± 0.39) and lowest value in diet 3 (11.81g ± 0.78). Specific growth rate (SGR) differed significantly (p<0.05), ranging from 1.49%day-1 ± 0.07 in diet 3 to 2.14%day-1 ± 0.02 in diet 1.

The result indicates that Diet 1 (0% replacement level) gave the best growth indices, followed by diet 5 (100% replacement level). This shows that thermally processed toad meal diets gave less growth performance indices than fishmeal; however, the values were still within acceptable limits when compared with other authors (Tiamiyu et al., 2013; Oso and Iwalaye, 2014). This is in contrast with the reports of Bekibele et al. (1995) who reported that diets of toad meal processed thermally at 1030c gave slightly better growth performance indices than fishmeal diets. Replacement of fishmeal with thermally processed toad meal indicated that their synergic effect was less than their individual effects. This is revealed as equal amount (50:50) of thermally processed toad meal and fishmeal gave the least growth parameters when compared to other replacement levels. This observation is similar to the reported work of Bekibele et al. (1995) who replaced fishmeal with thermally processed toad meal and observed that the replacement level of 50:50 gave the least growth parameter. Falaye et al. (2012) in a work involving the replacement of fishmeal with toad meal processed at 70^{0}C, observed that the least growth parameters was obtained at 50% replacement level.

Table 4 depicts the carcass analysis of *Clarias gariepinus* fed experimental diets of thermally processed toad meal as replacement for fishmeal. The results indicate that there were significant differences (P<0.05) between the initial carcass analysis and the final carcass analyses of all the treatments for all the parameters. Crude protein content of the carcasses of *Clarias gariepinus* fed experimental diets of thermally processed toad meal as replacement for fishmeal differed significantly (p<0.05), ranging from 10.37 ± 0.12 (initial) to 18.03 ± 0.28 (diet 1). This result corroborate that of the growth indices by revealing that fish fed diet 1 (0% replacement level) retained the highest protein in their carcasses.

Table 5 shows the mean water quality parameters measured during the experimental feeding of *Clarias gariepinus* juveniles with diets of thermally processed toad meal as replacement for fishmeal. The result reveals that there were no significant differences (p>0.05) among the different treatments for all parameters tested.

Table 1: Gross composition of experimental diets of thermally processed toad meal as replacements for fishmeal.

Ingredients	Diet 1 (0:100)	Diet 2 (25:75)	Diet 3 (50:50)	Diet 4 (75:25)	Diet 5 (100:0)
Thermally processed toad meal	0.00	7.50	15.00	22.50	30.00
Fishmeal	30.00	22.50	15.00	7.50	0.00
Soybean meal	39.13	35.75	32.36	28.98	25.60
Maizemeal	22.87	26.25	29.64	33.02	36.40
Vitamin and mineral premix	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Oil	3.00	3.00	3.00	3.00	3.00
Starch (as binder)	4.00	4.00	4.00	4.00	4.00
Total	100.00	100.00	100.00	100.00	100.00

Table 2: Proximate composition of experimental diets of thermally processed toad meal as replacement for fishmeal.

Parameters	Diet 1 (0:100)	Diet 2 (25:75)	Diet 3 (50:50)	Diet 4 (75:25)	Diet 5 (100:0)	**LSD**
Crude protein (%)	35.67 ± 0.43[a]	35.35 ± 0.35[a]	36.22 ± 0.25[a]	35.91 ± 0.1[a]	35.59 ± 0.19[a]	1.04
Ether extract (%)	9.19 ± 0.13[b]	8.99 ± 0.08[ab]	8.44 ± 0.23[a]	9.59 ± 0.08[b]	9.41 ± 0.25[b]	0.60
Ash (%)	10.35 ± 0.15[a]	12.59 ± 0.48[b]	12.76 ± 0.20[b]	10.98 ± 0.03[a]	13.43 ± 0.55[b]	1.25
Crude fibre (%)	9.06 ± 0.18[d]	4.40 ± 0.15[a]	8.98 ± 0.06c[d]	7.25 ± 0.11[b]	8.55 ± 0.18[c]	0.51
Moisture (%)	4.22 ± 0.09[c]	3.27 ± 0.13[b]	4.02 ± 0.07[c]	3.52 ± 0.13[b]	2.61 ± 0.07[a]	0.36
NFE (%)	31.52 ± 0.62[bc]	35.72 ± 0.72[d]	29.59 ± 0.66[a]	32.76 ± 0.12[c]	30.62 ± 0.23[ab]	1.93

Means on the same row with different superscripts are significantly different ($p<0.05$)

Table 3: Growth Performance of Clarias gariepinus juveniles fed experimental diets of thermally processed toad meal as replacement for fishmeal.

Parameters	Diet 1 (0:100)	Diet 2 (25:75)	Diet 3 (50:50)	Diet 4 (75:25)	Diet 5 (100:0)	LSD
MIW (g)	9.03 ± 0.03[a]	9.04 ± 0.03[a]	9.07 ± 0.01[a]	9.02 ± 0.02[a]	9.00 ± 0.01[a]	0.07
MFW (g)	29.85 ± 0.42[c]	25.70 ± 0.36[b]	20.88 ± 0.77[a]	24.31 ± 0.43[b]	26.41 ± 0.79[b]	2.12
MWG (g)	20.83 ± 0.39[d]	16.66 ± 0.33[bc]	11.81 ± 0.78[a]	15.29 ± 0.41[b]	17.41 ± 0.79[c]	2.09
%MWG	230.80 ± 3.68[d]	184.30 ± 3.12[bc]	130.20 ± 8.68[a]	169.50 ± 4.07[b]	193.50 ± 8.72[c]	22.49
SGR ($\%day^{-1}$)	2.14 ± 0.02[c]	1.87 ± 0.02[b]	1.49 ± 0.07[a]	1.77 ± 0.03[b]	1.92 ± 0.05[b]	0.15
FCR	1.96 ± 0.01[a]	2.14 ± 0.02[a]	2.60 ± 0.13[b]	2.15 ± 0.05[a]	2.11 ± 0.06[a]	0.25
PER	1.60 ± 0.01[c]	1.48 ± 0.02[b]	1.21 ± 0.06[a]	1.46 ± 0.03[b]	1.48 ± 0.04[bc]	0.14
ANPU	26.65 ± 0.74[d]	23.24 ± 0.23[bc]	20.93 ± 0.21[a]	22.56 ± 0.58[ab]	24.94 ± 0.70[cd]	1.93
%Survival	95.00 ± 0.00[b]	97.50 ± 0.00[b]	87.50 ± 2.50[a]	85.00 ± 2.50[a]	85.00 ± 0.00[a]	5.75

Means on the same row with different superscripts are significantly different ($p<0.05$)

Keys: MIW = mean initial weight, MFW = mean final weight, MWG = mean weight gain, %MWG= percent mean weight gain
SGR = specific growth rate, FCR = feed conversion ratio, PER = protein efficiency ratio, ANPU = Apparent Net Protein Utilization

Table 4: Carcass analysis of *Clarias gariepinus* juveniles fed experimental diets of thermally processed toad meal as replacement for fishmeal.

Parameters	Initial	Diet 1 (0:100)	Diet 2 (25:75)	Diet 3 (50:50)	Diet 4 (75:25)	Diet 5 (100:0)	LSD
Crude protein (%)	10.37 ± 0.12[a]	18.03 ± 0.28[e]	17.10 ± 0.07[cd]	15.98 ± 0.14[b]	16.66 ± 0.09[c]	17.24 ± 0.04[d]	0.49
Ether extract (%)	5.34 ± 0.25[a]	11.41 ± 0.07[c]	11.10 ± 0.25[c]	11.00 ± 0.03[c]	11.24 ± 0.08[c]	6.79 ± 0.24[b]	0.62
Ash (%)	2.05 ± 0.05[a]	3.05 ± 0.05[b]	4.16 ± 0.07[d]	3.85 ± 0.05[c]	3.25 ± 0.06[b]	3.69 ± 0.08[c]	0.21
Crude fibre (%)	0.80 ± 0.04[a]	1.20 ± 0.03[b]	1.41 ± 0.05[c]	1.23 ± 0.12[b]	1.45 ± 0.03[c]	1.70 ± 0.01[d]	0.11
Moisture (%)	74.66 ± 0.30[d]	64.72 ± 0.07[ab]	64.88 ± 0.16[ab]	65.45 ± 0.06[b]	64.36 ± 0.11[a]	66.88 ± 0.48[c]	0.86
NFE (%)	6.80 ± 0.16[e]	1.59 ± 0.26[a]	1.34 ± 0.14[a]	2.50 ± 0.03[b]	3.05 ± 0.15[c]	3.69 ± 0.12[d]	0.55

Means on the same row with different superscripts are significantly different ($p<0.05$)

Table 5: Mean water quality parameters measured during the experimental feeding of Clarias gariepinus juveniles with diets of thermally processed toad meal as replacement for fishmeal.

Parameters	Diet 1 (0:100)	Diet 2 (25:75)	Diet 3 (50:50)	Diet 4 (75:25)	Diet 5 (100:0)	**LSD**
Electrical Conductivity (µS)	724.00 ± 5.00a	729.50 ± 12.00a	750.20 ±13.25a	716.00 ± 14.00a	733.80 ±15.25a	35.81
Total Dissolved Solids (ppm)	361.20 ± 12.75a	363.80 ± 13.75a	375 .00± 15.00a	358.00 ± 12.00a	366.50 ± 8.00a	17.41
pH	7.72 ± 0.12a	7.72 ± 0.22a	7.66 ± 0.13a	7.70 ± 0.12a	8.23 ± 0.49a	0.80
Temperature (^{0}C)	26.80 ± 0.23a	26.88 ± 0.13a	26.45 ± 0.10a	26.70 ± 0.05a	26.42 ± 0.28a	0.55
Dissolved oxygen (mg/l)	3.90 ± 0.10a	4.05 ± 0.25a	3.95 ± 0.13a	3.95 ± 0.15a	4.05 ± 0.20a	0.33

Means on the same row with different superscripts are significantly different (p<0.05)

CONCLUSION

Thermally processed toad meal could be a fair enough replacement for fish meal at 100% replacement level because its growth indices were only a little lower than that of fishmeal. However, further investigation of the toxin contents of this toad meal is recommended to ascertain if thermal processing method have reduced or eliminated the toad toxin, before subsequent inclusion in fish diets.

Disclosure statement

No potential conflict of interest was reported by the author.

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