Octa Journal of Biosciences
International peer-reviewed journal
Octa. J. Biosci. Vol. 3(2):79-82

ISSN 2321 – 3663
Dec. 2015

Octa Journal of Biosciences

journal homepage: www.sciencebeingjournal.com

Effects Of Different Processing Methods On The Proximate Composition And Amino Acid Profile Of Toad (*Bufo regularis*)

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ARTICLE INFO

Received 16 Sept. 2015
Revised 19 Oct. 2015
Accepted 30 Nov. 2015
Available online 30 Dec. 2015

Keywords: Fish, Toadmeal, Ferment, Skinned, Deglanded Toadmeal

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ABSTRACT

The effects of various processing methods on the proximate composition and amino acid profile of toads were investigated. Toads were collected, weighed, gutted and processed differently into meals as whole toadmeal (WTM), thermally processed toadmeal (TTM), skinned and deglanded toadmeal (SDTM) and fermented toadmeal (FTM). The differently processed toadmeals were analysed for proximate composition and amino acid profile. The results showed that crude protein content differed significantly ($p<0.05$), ranging from from 43.56 ± 0.17 to 58.22 ± 0.67 with FTM having the lowest crude protein content and SDTM having the highest. Moisture content ranged from 2.55 ± 0.05 in SDTM to 3.75 ± 0.05 in WTM and the difference was statistically significant ($p<0.05$). The results also revealed that amino acids of the differently processed toadmeal differed significantly ($p<0.05$). Lysine was highest in SDTM (6.94 ± 0.17) and lowest in FTM (5.47 ± 0.45). The values for methionine ranged between 1.87 ± 0.26 and 2.30 ± 0.23 with FTM having the lowest and SDTM recording the highest value and the differences between treatments were significant ($p<0.05$). The results depicted that SDTM gave the best amino acid profile while fermented toadmeal had the least. Values for all the essential amino acids were highest in SDTM and lowest in FTM except for phenylalanine which was highest in TTM. The amino acid profile of some of the toadmeals especially that of SDTM and TTM were comparable to that reported for fishmeal and superior to some other unconventional feedstuffs. Although skinning and deglanding method of processing gave comparably the best results in terms of proximate composition and amino acid profiles, however, there is need for further investigations to ascertain the detoxifying effects of these processing methods on the toxin of toads, before studies on feeding trials at different inclusion levels can be conducted.

INTRODUCTION

In aquaculture nutrition, the quality and quantity of protein available in fish diet is of paramount importance because, fish unlike terrestrial farm animals is able to derive more metabolizable energy from the catabolism of proteins than from carbohydrates, and thus, they require higher dietary protein than other animals (Tacon, 1987; Craig and Helfrich, 2009). Therefore, the growth and nutritional status of fish depends on the quality and quantity of dietary protein in its meal.

Fishmeal is the most preferred protein source in fish diets due to its excellent amino acid profile. However, the supply of fishmeal is not adequate to sustain the growing aquaculture industry and the competitive demand from other livestock feed industries (Naylor et al, 2000). This position makes fishmeal expensive, increasing the recurrent cost due to feed in fish production.

The search for alternative protein sources to fishmeal is a priority research in aquaculture nutrition because of concerns regarding the future availability of fishmeal for incorporation into fish diets (Mondal, et al., 2008). In the choice of these alternative feed ingredients, availability, nutrient composition, easy accessibility and lack of competition with other consumers are key factors. Several researches have been directed towards alternative protein feed ingredients of both plant and animal origin that can supply comparable nutritional value at lesser cost. However, attempts to use these feedstuffs to replace fishmeal in fish diets have met with variable success due to factors such as the protein content, amino acid profile and anti nutritional factors in these feeds among others (Ogunji, 2004).

Toad meal processed from toads of the family Bufonidae (*Bufo regularis*) has been identified to have good prospects as an unconventional protein feedstuff (Bekibele, et al., 1995; Falaye, et al., 2012). Aside from the fact that toads are abundant especially during the rainy seasons when they can be harvested at street corners, behind houses, bushes and stagnant waters, they have no competition from man and very few animals are known to prey on them. However, their use as meal for fish diets is limited by their possession of poison glands containing toxic substances (Sciani, et al., 2013).

Processing methods employed during most feed preparations are aimed at detoxification of toxins and reduction of anti Nutritional Factors (ANFs) in the feedstuffs. This could be done by boiling/cooking, fermenting with/without enzymes, soaking, heating, roasting, blanching, extruding among other methods (Akpodiete and Okagbare, 1999; Isikwenu and Bratte, 1999; Obun et al., 2005; Tiamiyu et al., 2007). However, these different methods of feedstuffs processing could also have diverse effects on the proximate composition and on the amino acid profile of the feedstuffs.

Proximate composition shows the various nutritional components of a feedstuff while the amino acid profile gives the various amino acids that make up the protein present in the feedstuff. This is of great importance because fish require all the essential amino acids (EAAs) in their diet for proper physiological functioning, growth and maintenance of the body (Craig and Helfrich, 2009). The deficiency in one or more of these EAAs has been reported to result in growth reduction, pathological symptoms or even death.

Therefore, this work aimed at investigating the effects of different processing methods on the proximate composition and amino acid profile of toads (*Bufo regularis*).

MATERIALS AND METHODS

Processing of toadmeal

Toads used in this research work were harvested in streams along international market road, Makurdi, Nigeria and were killed by knocking them to unconsciousness and processed in four different methods as follows:

Whole toad processing

The toads were weighed, gutted, washed and oven dried at 60^{0}C to a constant weight.

Skinning and deglanding

The toads were weighed, skinned and the parotid glands removed. The processed toads were thereafter washed and oven dried at 600C to a constant weight.

Thermal processing

The toads were weighed, gutted, washed and oven dried at 100^{0}C to a constant weight.

Fermentation

The toads were weighed, gutted, washed and fermented in earthen pots with lids for 3 days. The fermented toads were thereafter oven dried at 60^{0}C to a constant weight.

Analysis of proximate composition

The proximate compositions of the differently processed toadmeal were determined using AOAC (2005) standard method.

Determination of Amino Acid Profile

The Amino Acid profile was determined using methods described by Benitez (1989). The samples were dried to constant weight, defatted, hydrolyzed with 6NHCL, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample

Amino Acid Analyzer (TSM). During hydrolysis with 6NHCL, tryptophan was destroyed and therefore, in determining tryptophan, hydrolysis was performed using sodium hydroxide (NaOH).

Data analysis

Statistical analysis of data collected involved descriptive statistics and results were presented as means and standard error of means. Data collected were also subjected to analysis of variance where the means were separated by least significant difference using Genstat package edition 12.

RESULTS AND DISCUSSION

Table 1 shows the proximate composition of the differently processed toadmeals: skinned and deglanded toadmeal (SDTM) thermally processed toadmeal (TTM), fermented toadmeal (FTM) and whole toadmeal (WTM). The crude protein content of the differently processed toadmeals differed significantly ($p<0.05$). The crude protein content ranged from 43.56 ± 0.17 to 58.22 ± 0.67 with FTM having the lowest crude protein content and SDTM having the highest. The high crude protein content in SDTM could be attributed to the reduction in other components such as moisture and crude fibre contents of the sample due to the removal of the skin and parotid gland. Crude protein was also high in TTM and this could be attributed to the effect of higher processing temperature (100^oc) which reduced other parameters such as moisture and crude fibre contents.

The low crude protein level in fermented toadmeal is likely due to the fact that protein was used up by microorganisms causing fermentation and also through the action of catalytic enzymes which are known to break down body tissues. Fermented product has been defined as any product which has undergone degradative changes through enzymatic or microbiological activity either in the presence or absence of salt (Essuman, 1992). The low crude protein level in fermented toadmeal in the present work is in agreement with the reports by several authors who have attributed the reduction in protein content of fermented products to the accelerating rate of protein catabolism by microorganisms in fermentation (Amano 1962; Anihouvi et al., 2006 and Koffi-Nevry, et al,. 2011; Faisal, et al., 2013). Similarly, Essuman (1992) identified that in the course of fermentation, there is breakdown of protein with subsequent formation of ammonia (NH3) indole, hydrogen sulphide, etc. However, other authors have observed an increase in the crude protein content of fermented products when compared to the unfermented ones (Ekpe, et al., 2007; Makanjuola and Ajayi, 2012 and Sani et al., 2013). They attributed the increase in crude protein content mostly to increased production of single cell proteins as a result of microbial proliferation. This variance could have been due to the difference in the methods employed in fermentation, additional materials or microorganisms introduced to aid fermentation and fermentation substrates used, taking into cognizance the fact that the authors mentioned previously reported on fermentation using animal substrates while those mentioned later used plant substrates.

Table 1: Proximate composition of the differently processed toadmeals

Parameters	SDTM	TTM	FTM	WTM	LSD
Crude protein (%)	58.22 ± 0.67^c	57.96 ± 0.06^c	43.56 ± 0.17^a	48.90 ± 0.545^b	1.72
Ether extract (%)	5.09 ± 0.27^c	5.02 ± 0.08^c	1.81 ± 0.12^a	4.07 ± 0.140^b	0.65
Ash (%)	23.59 ± 0.74^b	23.03 ± 0.42^b	15.32 ± 0.56^a	23.95 ± 0.05^b	2.00
Crude fibre (%)	0.36 ± 0.05^a	0.57 ± 0.02^{ab}	0.80 ± 0.14^b	1.19 ± 0.06^c	0.32
Moisture (%)	2.55 ± 0.05^a	2.59 ± 0.07^a	3.56 ± 0.13^b	3.75 ± 0.045^b	0.31
NFE (%)	10.20 ± 1.67^a	10.85 ± 0.37^a	34.96 ± 1.21^c	18.15 ± 0.055^b	4.12

Means on the same row with different superscripts are significantly different (p<0.05)
WTM = Whole toadmeal, TTM= Thermally processed toadmeal, SDTM = Skinned and deglanded toadmeal, FTM = Fermented toadmeal

Generally, the crude protein contents of all the samples were lower than those reported for this species by Falaye, et al. (2012) who reported a crude protein value of 63%. This difference could be due to spatial difference (Ibadan being a rain forest zone and Makurdi being a guinea savannah zone) or difference in the seasons in which toads used in both works were caught, as seasonal changes can affect proximate composition. The values for crude protein reported by Bekibele et. al. (1995) (59.29%) and Esonu (2012) (59.06) are close to that recorded for skinned, deglanded (58.22%) and thermally processed (57.96%) toadmeals in this work. However, the crude protein contents of the different processed toadmeals in this work are higher than that which is reported by Ojewola and Udom (2005), who reported a crude protein value of 45.72%. This variation may be due to the difference in toad species used even as the authors did not specify the species of toad used for the analysis.

The Moisture content of the differently processed toadmeals differed significantly ($p<0.05$), ranging from 2.55 ± 0.05 in SDTM to 3.75 ± 0.05 in WTM. The reduction in moisture content in SDTM could have been due to the removal of the fibrous skin and parotid gland whose interstitial water molecules are more difficult to release. This is further explained by the reduction in fibre content in SDTM and it is in agreement with Ekpe, et al., (2007) who reported that high dietary fibre indicates the presence of reasonable quantity of trapped water which is held by the hydrophilic polysaccharides of the fibre. The reduction in moisture content of TTM could be attributed to the effect of the high temperature employed during processing which released interstitial water molecules.

The Crude fibre content also differed differently ($p<0.05$) in all the differently processed toadmeals. Crude fibre content was highest in WTM (1.19 ± 0.06) and lowest in SDTM (0.36 ± 0.05). Table 2 shows the amino acid profile of the variously processed toadmeals: SDTM, TTM, FTM and WTM. The amino acid profile of the variously processed toadmeals varied significantly ($p<0.05$). The level of arginine in the differently processed toad meal differed significantly ($p<0.05$), ranging from 6.17 ± 0.22 in FTM to 8.00 ± 0.14 in SDTM. The values of arginine for all the treatments were higher than those reported for fishmeal (3.82) (NRC, 1993), bloodmeal (3.75) (NRC, 1993), *Agama agama* (4.68 ± 0.58) (Tiamiyu, et al., 2014), housefly maggotmeal (5.80) (Aniebo, et al., 2009) and Winged reproductive termite (4.32) (Solomon, et al., 2007).
The level of Lysine in the variously processed toadmeals varied significantly ($p<0.05$), with SDTM (6.94 ± 0.17) recording the highest value and FTM (5.47 ± 0.45) having the lowest value. The values for lysine in all the different processed toadmeals were higher than those reported for fishmeal (4.72) (NRC, 1993) and *Agama agama* (4.10 + 0.87) (Tiamiyu, et al., 2014), but were lower than that of bloodmeal (7.45) (NRC, 1993) and comparable with those for Wing reproductive termite meal (6.15) (Solomon, et al., 2007) and Housefly maggotmeal (6.04) (Aniebo, et al., 2009).

The level of methionine in the differently processed toadmeals varied significantly ($p<0.05$). The values for methionine ranged between 1.87 ± 0.26 and 2.30 ± 0.23 with FTM having the lowest and SDTM recording the highest value. The values of methionine for SDTM and TTM were comparable with those reported for fishmeal (2.3) (NRC, 1993), Bloodmeal (2.32) (NRC, 1993), Wing reproductive termite meal (2.24) (Solomon, et al., 2007) and Housefly maggot meal (2.28) (Aniebo, et al., 2009). However, the values of methionine for all the different processed toadmeals were higher than that of Agama agama lizard (1.00 + 0.58) (Tiamiyu, et al., 2014).

Isoleucine was highest in SDTM (3.20 ± 0.50) and lowest in FTM (2.29 ± 0.11) with statistical difference ($p<0.05$) between treatments. The level of tryptophan in the differently processed toadmeals varied insignificantly ($p>0.05$) ranging from 0.62 ± 0.49 in FTM to 0.73 ± 0.17 in SDTM.

The results of the present study reveals that skinned and deglanded toadmeal gave the best amino acid profile. The best amino acid profile given by SDTM is concurred by its high crude protein content. The values for the essential amino acids for all the different processed toad meals reported in this work were higher than the amino acid requirements reported for channel catfish, rainbow trout, pacific salmon, common carp and tilapia (NRC, 2011). The values for arginine, histidine, leucine, lysine, threonine and valine for all the different processed toadmeals were higher than those recommended for African catfish diet (Robinson, et al., 2006).The values of isoleucine for SDTM and TTM were higher than the recommended value for African catfish (2.6), while the values for WTM was comparable and that of FTM was lower. For methionine, the values for SDTM and TTM were comparable, while the values for WTM and FTM were lower than that which is recommended for the African catfish (2.30) (Robinson, et al., 2006).

CONCLUSION

Toad meal processed by skinning and deglanding stood out as the best amongst other processing methods with high crude protein content and good amino acid profile which is comparable with that of fishmeal and superior to those of some unconventional feedstuffs. However, there is need for further investigations to ascertain

Parameters	WTM	TTM	SDTM	FTM	LSD
EAAs					
Arginine	7.32 ± 0.23[b]	7.97 ± 0.16[c]	8.00 ± 0.14[c]	6.17 ± 0.22[a]	0.24
Histidine	2.45 ± 0.25[b]	2.49 ± 0.34[b]	2.50 ± 0.20[b]	1.95 ± 0.14[a]	0.31
Isoleucine	2.60 ± 0.18[b]	3.18 ± 0.42[c]	3.20 ± 0.50[c]	2.29 ± 0.11[a]	0.20
Leucine	7.36 ± 0.35[b]	7.72 ± 0.44[c]	7.86 ± 0.18[c]	6.42 ± 0.69[a]	0.15
Lysine	5.92 ± 0.16[b]	6.92 ± 0.35[c]	6.94 ± 0.17[c]	5.47 ± 0.45[a]	0.32
Methionine	2.20 ± 0.52[b]	2.29 ± 0.36[b]	2.30 ± 0.23[b]	1.87 ± 0.26[a]	0.14
Phenylalanine	4.72 ± 0.25[b]	4.82 ± 0.31[b]	4.80 ± 0.64[b]	3.73 ± 0.29[a]	0.14
Threonine	3.77 ± 0.34[b]	4.10 ± 0.17[bc]	4.20 ± 0.13[c]	2.42 ± 0.24[a]	0.39
Valine	3.28 ± 0.32[a]	4.10 ± 0.29[b]	4.22 ± 0.29[b]	3.20 ± 0.55[a]	0.14
Tryptophan	0.68 ± 0.51[a]	0.72 ± 0.38[a]	0.73 ± 0.17[a]	0.62 ± 0.49[a]	0.14
NEAAs					
Alanine	4.20 ± 0.23[b]	4.63 ± 0.29[c]	4.68 ± 0.14[c]	3.47 ± 0.23[a]	0.36
Aspartic acid	9.67 ± 0.29[b]	10.30 ± 0.35[c]	10.19 ± 0.2[bc]	8.00 ± 0.13[a]	0.62
Cystine	0.61 ± 0.43[a]	0.67 ± 0.46[a]	0.68 ± 0.53[a]	0.53 ± 0.52[a]	0.16
Glutamic acid	12.24 ± 0.34[b]	13.73 ± 0.15[c]	13.78 ± 0.17[c]	11.00 ± 0.14[a]	0.20
Glycine	4.58 ± 0.17[b]	4.94 ± 0.48[c]	4.96 ± 0.14[c]	3.28 ± 0.18[a]	0.21
Proline	2.66 ± 0.12[b]	3.16 ± 0.26[c]	3.17 ± 0.23[c]	1.85 ± 0.18[a]	0.47
Serine	3.53 ± 0.14[b]	3.87 ± 0.46[c]	3.85 ± 0.19[c]	2.85 ± 0.29[a]	0.18
Tyrosine	2.63 ± 0.40[b]	3.12 ± 0.29[c]	3.18 ± 0.17[c]	2.33 ± 0.76[a]	0.15

Means on the same row with different superscripts are significantly different ($p<0.05$)
WTM = Whole toadmeal, TTM= Thermally processed toadmeal, SDTM= Skinned and deglanded toadmeal, FTM = Fermented toadmeal, EAA=Essential amino acids, NEAA= Non-essential amino acids.

investigations to ascertain the effects of these processing methods on the toxins of toads. It is after such an investigation that further studies on feeding trials at different inclusion levels can be conducted and subsequent recommendation to farmers for incorporation into fish diets as the case may be.

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