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EVALUATION OF TOXICOLOGICAL AND NUTRITIONAL PARAMETERS OF DETOXIFIED DEFATTED DIETARY CASTOR SEEDS IN FISH NUTRITION

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ABSTRACT

Castor seed de-oiled into cake (CSC) was differently processed by boiling, roasting, boiling & roasting, decorticated lyle-treated, boiling & fermenting and fed at 10 % in diets to 180 juvenile *Clarias gariepinus* (Catfish) in a 4-week feeding trial to assay its nutritional and toxicological impact using performance and some bio-data indices. Treatment methods other than boiling improved performance traits and the other measured bio-data such as specific growth rate (SGR), protein efficiency ratio (PER), net protein utilization (NPU), nitrogen metabolism (NM) ($p < 0.05$). Some processing method(s) (roasting) gave superior results than the others ($p < 0.05$) relative to the conventional diet. Boiled dietary CSC however, produced the poorest results compared to the other methods and the control diet ($p < 0.05$). Roasted CSC in diet gave the best result among the dietary treatments ($p < 0.05$) hence it was recommended that CSC be processed by roasting before inclusion in diet at 10 % for fish.

Keyword : Castor seed cake, *Clarias gariepinus*, performance, bio-data indices.

ÉVALUATION DES PARAMÈTRES TOXICOLOGIQUES ET NUTRITIONNELS DU TOURTEAU DE RICIN DÉTOXIFIÉ DANS L'ALIMENTATION DES POISSONS

RÉSUMÉ

Le tourteau de graine de ricin ayant subi différentes méthodes de traitements (ébullition, torréfaction, ébullition-torréfaction, traitement par lessivage des graines décortiquées, ébullition-fermentation) a été incorporé à 10 % dans l'aliment de 180 jeunes *Clarias gariepinus* (poisson-chat) pendant 4 semaines en vue d'évaluer son effet nutritionnel et toxicologique à partir des performances de croissances et d'autres paramètres biométriques. Les méthodes de traitements autres que l'ébullition ont amélioré de manière significative ($p < 0.05$) les performances ainsi que les paramètres biométriques tels que : le taux spécifique de croissance (SGR) ; le taux d'efficience protéique (PER) ; l'utilisation protéique net (NPU) ; métabolisme protéique (NM) et le taux de survie (SR). Certaines méthodes de traitement (comme la torréfaction) ont donné des résultats supérieurs à d'autres, par rapport à l'aliment conventionnel. Cependant, la ration à base de tourteaux de graines de ricin bouillies, comparée aux autres traitements et à la ration témoin, a produit les plus faibles résultats ($p < 0.05$). De tous les traitements, la ration à base de tourteaux de graines de ricin torréfiés est celle qui a donné les meilleurs résultats et de ce fait, il est recommandé que les tourteaux de graines de ricin soient torréfiés avant d'être incorporés à 10 % dans l'alimentation des poissons-chats.

Mots-clés : Tourteau de graine de ricin, *Clarias gariepinus*, performance, paramètres biométriques.

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INTRODUCTION

Plants have been used for centuries as sources of food and feedstuffs for man and animals both domesticated animals and those on free range (Shafaer *et al.*, 2011 ; El-Said & Al-Barak, 2011). It is a well-known phenomenon that plants are biosynthetic laboratory for chemical compounds that are used for food such as carbohydrates, proteins, fats, vitamins, minerals and those that elicit toxic and/or physiological effects on the body when consumed in amounts above the normal threshold. According to Aletor (1993), there are numerous anti-nutritional or toxic factors that are very significant in plants used for human foods and animal feeds namely enzymes (carbohydrate, protein, plasmin, elastase) inhibitors, plant enzymes (urease, lipoxygenase), cyanogenic glycosides (phaseolunatin, dhurrin, linamarin, lutaustralin), goitrogens (flavones and genistein), saponins (*soya sapogenin*), gossypol from Gossypium species like cotton, tannins (condensed and hydrolysable tannins), amino acid analogues (BOAA, DAP, mimosine, N-methyl-1-alanine), alkaloids (solanine and chaconine), anti-metals (phytates and oxalates), anti-vitamins (anti-vitamins A, D, E, K and B12), Favism factors, haemagglutinins/lectins (concanavalin A, ricin, ricinine), the list is endless. The latter chemical compounds are known as secondary metabolites while the former (carbohydrates, protein, fat) are known as the primary metabolites of the plants kingdom.

Castor seeds evaluated in this work as alternative protein to the scarce and expensive orthodox plant proteins (groundnuts, soybeans) harbour both the primary and secondary metabolites. Castor seeds are produced by the castor plant, *Ricinus communis*, which is widely cultivated in the temperate, tropical and sub-tropical countries mainly as source of plant/vegetable oil. The seeds contain about 50 %. The cake or meal left after oil extraction has high protein content of 39 %, crude fiber 18 %, 7.5 % mineral matter, 3 % soluble carbohydrate and 40 % ether extract (Devendra, 1988). Notwithstanding these nutritional merits of the cake, its application in nutrition of farm animals is not popular due to the presence of some phytotoxins such as ricin (a highly toxic protein), ricinine (a relatively harmless alkaloid), hydrocyanic acid, an extremely potent allergen considered toxic to both man and animals (Sevkovic & Popoviv, 1966). The seed residue after oil extraction is considered as waste and discarded. It is for the reason to source for cheap alternative feed sources that this experiment attempted the detoxification of castor seed cake (CSC) for use in nutrition of fish.

MATERIALS AND METHODS

Castor seeds were obtained from a commercial farm at Sango within Ilorin metropolis. The raw seed were pressed using a mechanical press to remove some oil before the chemical extraction with N-hexane to further extract the oil. N-hexane was thoroughly mixed with the CSC meal in the ratio of 2:1 and left for 4h to extract after which the mixture was decanted and the residue taken in a muslin cloth, tied and placed under a perforated manual screw jack oil extractor. The jack was frequently adjusted to ensure an oil-free cake. The cake obtained was sun-dried for many days before subjecting to the five different dietary treatments used in this experiment viz : cooking, roasting, boiling & roasting, lyle-treated decorticated CSC, cooking & fermenting.

Cooking treatment

1kg CSC was placed in a woven sac and immersed in 2-litres of boiling water maintained at 100^{o}C for 1h. The sac was removed and water pressed out of the dough and sun-dried for three days prior to inclusion in the diet.

Roasting

Dry heating was carried out by frying 1 kg CSC in a heated container for 20 minutes. The roasted cake was sun-dried before mixing in the diet.

Roasting & boiling

2 kg CSC was roasted and boiled as described above and sun-dried to constant weight for 3-days before incorporation in the diet.

Lyle treatment

Plantain peels were burnt to ashes and the ash soaked in water for 48h to extract the *lyle*. The filtrated (lyle) was decanted and strained via a muslin cloth to obtain a strong alkali of about pH 12. Decorticated castor seeds were subjected to oil extraction as described before to obtain the CSC which was soaked in the *lyle* for one day before drying for inclusion in diet mixture.

Cooking & fermenting

10 kg CSC was boiled for an hour and subjected to lactic fermentation following the procedures of Annongu *et al* (1996).

Experimental Diets, Design, Feeding trial and Management

Six (6) diets with approximately 40 % crude protein to meet NRC (1993) requirements for fish were formulated as shown on Table 1.

Table 1a. Composition of the experimental diets on as fed basis (kg/100kg feed)

Diets	Control	Boiled CSC	Roasted CSC	B & RCSC	LT CSC	B & FCSC
Ingredients						
Maize	21.50	17.50	17.50	17.50	17.50	17.50
Soybeanmeal	50.00	44.30	44.30	44.30	44.30	44.30
PCSC*	0.00	10.00	10.00	10.00	10.00	10.00
Blood meal	18.00	17.70	17.70	17.70	17.70	17.70
Premix	2.00	2.00	2.00	2.00	2.00	2.00
DLmethionine	0.75	0.75	0.75	0.75	0.75	0.75
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vegetable oil	3.25	3.25	3.25	3.25	3.25	3.25
Starch	3.25	3.25	3.25	3.25	3.25	3.25
Lysine	0.75	0.75	0.75	0.75	0.75	0.75
Total	100.0	100.0	100.0	100.0	100.0	100.0

**PCSC, processed castor seed cake.*

Table 1b. Analyzed nutrients content of the experimental diets (%DM)

Diets	Dry matter	Total ash	Crudeprotein	NFE*	Crude fat	Crudefiber
Raw CSC	93.60	9.00	41.10	34.40	3.90	5.20
Boiled CSC	93.69	9.45	42.10	29.29	7.25	4.60
Roasted CSC	92.40	9.87	40.13	29.20	8.85	4.75
B & RCSC	94.10	9.75	41.41	33.20	6.20	4.50
LT CSC	93.80	11.10	41.50	33.65	4.20	3.40
B & FCSC	94.35	9.93	40.20	34.10	4.45	4.70

**NFE, nitrogen free extract/soluble carbohydrate*

The control diet had maize-soybeans as basic ingredients while diets 2, 3, 4, 5 and 6 contained 10 % inclusion of cooked, roasted, cooked & roasted, *lyle* treated and cooked-fermented CSC respectively. The objective was to obtain the best treatment(s) that could detoxify the CSC phytotoxins. A completely randomized design was used for the experiment and each dietary treatment was replicated thrice containing equal number of juvenile *Clarias gariepinus* (Cat fish). The 180 young cat fish were randomly allotted to the 18 large plastic bowls filled with 25 liters of borehole (well) water. Nets were used to cover the bowls' tops to prevent entrance of foreign materials and the fish from jumping out of the bowls. The bowls were arranged on Tables following a single factor design model. The fish received twice daily (8 am and 4 pm) 4 % of feed according to their body weight and the supply of the experimental feed was increased in response to the increase in weight of the fish.

The due management practices followed as the experiment lasted include change of water supplied to the bowls to fresh one every two days, removal of the ort (left over feed) and dirt, maintenance of the water quality physico-chemical parameters for good health of the fish using Hannah's complete water analysis kit. Besides, temperature and pH in the bowls were measured twice and once daily with a Hannah thermometer and coning pH meter respectively. While the experiment lasted, data were collected on feed intake, weight gain and feed efficiency (F/G) was determined by calculation. Survival rate was examined daily. Other biological data investigated in the course of the experiment included specific growth rate (SGR), net protein utilization (NPU), protein efficiency ratio (PER), protein-nitrogen metabolism (NM) and percent survival rate (SR). These indices were determined using the formulae:

$SGR = (\ln W2 - \ln W1) / (T2 - T1)$.

Where W2 = weight at time T2 day and W1 = weight at time T1 day.

NPU was expressed as apparent NPU and ANPU was computed as:

ANPU = Carcass protein (g) x 100 / Protein fed (g)

PER = Body weight gain of fish (g) / Crude protein fed (g)

$NM = 0.549 (b - a)h / 2$

Where 0.549 is the factor used for daily endogenous nitrogen losses in ruminants. However, this value is much as twice that obtained in monogastric animals such as *Clarias gariepinus* hence the denominator of 2.

b= Final body weight of the fish (g)

a = Initial body weight of the fish (g)

h = The number of experimental days.

Percent SR = (Initial number of fish stocked – Mortality) x 100 / Initial number of fish stocked

NB: *ANPU, apparent net protein utilization.

Chemical analyses

The chemical composition of the differently processed CSC, cooked, roasted, roasted & cooked, *lyle*-treated , cooked & fermented and the proximate analysis of the experimental diets were conducted following the standard methods of A. O. A. C. (1990).

Statistical analysis

Data obtained were analyzed by analysis of variance (ANOVA) using the model for a completely randomized design (Steel & Torrie, 1980). Significant differences between treatment means were further determined by Ducan multiple range test (Duncan, 1955).

RESULTS

Analyses of the chemical composition of CSC treated by the various methods are shown on Table 2. Processing CSC increased the nutrients, crude protein fat and mineral matter while crude fiber and dry matter contents did not differ from those of the virgin CSC. Table 3 presents data on the performance indices of fish fed the variously processed CSC. Feed consumption on CSC treated by roasting (T3) was comparable with the reference diet ($p > 0.05$) while feed intake on roasted and boiled CSC (T4), 1*yle*-treated CSC (T5) and boiled-fermented CSC based diet (T6) was better than that on the conventional diet ($p < 0.05$). On the other hand, CSC treated by boiling reduced feed intake relative to the control diet ($p < 0.05$). Body weight gain was highest on roasted CSC based diet (T3) followed by lyle-treated CSC diet (T5) while boiled & fermented CSC diet (T6) was comparable with the control diet ($p > 0.05$). Dietary roasted CSC gave the best feed conversion ratio (0.08) among the diets ($p < 0.05$). Survival rate on dietary boiled CSC was the least (40 %) compared with the other treatments which survival rate ranged between 73 – 93 %. Among the dietary treatments, roasting CSC before feeding in diet gave the highest specific growth rate (SGR), Protein efficiency ratio (PER), protein-nitrogen metabolism (NM) and net protein utilization (NPU) ($p < 0.05$) while processing by boiling produced the least results (Table 4) on these determinants ($p < 0.05$).

Table 2. Proximate composition of virgin and processed CSC (%DM).

Treatments	Dry matter	Mineral matter	Crude protein	NFE	Ether extract	Crude fiber
Raw CSC	96.98	7.14	35.43	24.88	25.10	4.43
BoiledCSC	93.51	6.16	42.00	13.50	28.45	2.40
RoastedCSC	92.80	4.40	36.31	13.24	35.50	3.35
Boiled-roasted CSC	93.65	6.75	37.41	21.10	24.20	4.20
Lyle-treated CSC	94.70	9.39	36.86	20.43	23.97	4.10
Boiled-fermented CSC	94.25	10.00	37.14	16.86	23.30	5.40

Table 3. Dietary performance of *C. gariepinus* fed differently treated CSC

Diets	Control	Boiled CSC	Roasted CSC	B&RCSC	LTCSC	B&FCSC	SEM
Feed intake(g/d)	3.38^{b}	1.21^{a}	3.33^{b}	4.70^{d}	4.80^{d}	4.00^{c}	0.02
Weight gain(g/d)	18.56^{c}	7.33^{a}	37.67^{e}	14.99^{b}	22.43^{d}	17.65^{c}	3.56
Feed efficiency(F/G)	0.18^{b}	0.16^{b}	0.08^{a}	0.31^{d}	0.21^{c}	0.22^{c}	0.14
SR (%)*	93.33	40.00	83.24	93.33	93.33	73.34	3.30

a-b-c-d-e : Treatment means in the same row not sharing common letters differed significantly ($p < 0.05$), *SR, survival rate, *B&R CSC, boiled & roasted CSC; LTCSC, lyle-treated CSC; B&F CSC, boiled & fermented CSC

Table 4. Aspects of biological indices of fish fed variously processed dietary CSC

Diets	Control	BoiledCSC	RoastedCSC	B&RCSC	LTCSC	B&FCSC	SEM
SGR	6.04^{b}	4.12^{a}	7.51^{d}	5.59^{b}	6.43^{c}	5.93^{b}	0.08
PER	15.72^{d}	7.97^{a}	28.28^{e}	13.38^{c}	11.71^{b}	11.12^{b}	0.09
NM	107.01^{d}	42.27^{a}	217.16^{f}	86.43^{b}	129.28^{e}	101.76^{c}	4.84
NPU	21.29^{d}	15.30^{a}	22.34^{e}	16.43^{b}	61.60^{f}	18.02^{c}	3.35

a-b-c-d-e-f : Means in the same row not sharing common superscripts are significantly different ($p < 0.05$).

Table 5 presented data on the average water quality and temperature magnitude which values were identical on all the treatment groups in the course of the experiment.

Table 5. Data on water quality and temperature highs in bowls used for the experimental fish

Replicates	Water pH	Water temperature (°C)
1A	7.00	26.00
B	7.40	26.00
C	7.40	26.50
2A	7.00	26.50
B	7.30	26.50
C	7.20	27.00
3A	7.10	28.50
B	7.20	26.50
C	7.30	26.50
4A	7.30	26.50
B	7.10	26.50
C	6.50	26.00
5A	7.10	27.00
B	7.30	26.50
C	7.10	26.50
6A	6.80	26.00
B	7.10	26.50
C	6.90	26.50

DISCUSSION

Proximate analysis of raw and processed CSC showed that treatments increased the contents of crude protein, ether extract and mineral matter. The increment in nutritional value following CSC treatments is of great nutritive advantage to the fed fish or other food animals. Dietary performance of the fish fed the differently treated CSC indicated that treatments improved performance of *C. gariepinus*. Highest improvement was recorded on CSC treated by roasting before inclusion in diet followed by *lyle*-treated and boiled-fermented. However, boiling CSC before incorporation in diet yielded poor performance traits relative to the control diet or the other test diets hence low feed intake, weight gain and poor feed efficiency of feed utilization coupled with the highest mortality on this diet (60 % mortality). Similarly, inferior or poor results were obtained on boiled CSC based diet using the other bio-data of SGR, PER, NPU and NM. Poor performance and values of SGR, PER, NPU and NM obtained on boiled CSC diet suggests that boiling treatment could not eliminate or reduce the *Ricinus communis* toxic phytochemicals namely ricin, ricinine, hydrocyanides, allergins, lectins. On the other hand, highest performance and the other bio-data values obtained on CSC treated by roasting may be that this method is feasible to deal with the *R. communis* toxins that usually hinder availability and utilization of nutrients in the fed animal. Past works (Huet, 1972) reported similar findings.

The similarity in data obtain in all the bowls on water quality and temperature magnitude among the treatment groups proved that no group of fish receiving any of the treatment was short-changed or disadvantaged by the environmental factors measured.

As conclusion, it is established in this study that best result could be obtained on performance from *C. gariepinus* if CSC is roasted before inclusion in diet at 10%. Further works to treat CSC by roasting and including beyond 10% are recommended.

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