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Investigation on Biochemically Processed Castor Seed Meal in Nutrition and Physiology of Japanese Quails

Research Article

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ABSTRACT

Native de-oiled and treated castor seed meal was subjected to proximate analysis and quantification of anti-nutrients (phytochemicals). Seed cake was treated by biochemical technique of solid state fermentation with *Aspergillus niger* and addition of calcium oxide (CaO) to give treated castor seed meal (TCSM). One hundred and twenty Japanese quails (*Coturnix coturnix japonica*) were fed four (4) iso-nitrogemous and iso-caloric diets containing 0, 2.5, 5.0 and 7.5% TCSM corresponding to the diet 1, 2, 3 and 4, respectively. While the feeding trial lasted for 56 days, feed and water were supplied *ad libitum*. Data on proximate composition showed that raw seed, defatted residue (cake) and the processed castor seed meal contained valuable nutrients like dry matter, crude protein, fat, fibre, mineral matter and soluble carbohydrate (NFE). Phytochemical quantification gave high levels of the anti-nutrients such as ricin, allergens, ricinine in the raw seed. However, levels of these phytochemicals were reduced by defatting and treatments of the cake meal by solid state fermentation, *A. niger* and CaO. Performance traits indicated decreases in feed intake, weight gain, growth and increases in mortality rates especially on the diet with the highest (7.5%) inclusion of TCSM compared to the control diet ($P<0.05$). Nutrients retentions on the test feedstuff were not comparable with values on the reference diet on soluble carbohydrate values which decreased with increasing CSM ($P<0.05$) relative to the control diet. In haematological parameters packed cell volume (PCV) and mean corpuscular hemoglobin (MCH) values on diets with TCSM were exceptionally high relative to the control diet ($P<0.05$). However, biochemical indices (serum protein, albumin, globulin, albumin:globulin ratio and alkaline phosphatase (ALP) activities were not influenced by dietary CSM ($P>0.05$). Enzyme activity of aspartate aminotransferase (AST) showed decreasing trend with increasing level of CSM in diets ($P<0.05$). Profiling electrolytes in the fed quails showed significant variations in concentrations of Ca^{++} and HCO_3^- on TCSM based diets ($P<0.05$) comparable with the control diet values. Conclusively, despite treating CSM by solid state fermentation with *A. niger* and CaO addition in this trial, TCSM addition still appears to induce toxic and deleterious effects on the quails. Subsequent works to enable inclusions at acceptable and higher levels after treatments are on-going.

KEY WORDS blood-composition, castor seed meal, Japanese quail, performance, solid-state-fermentation.

INTRODUCTION

Meeting the demand for animal protein requirement in developing countries of the world is becoming very difficult due to daily increment in human population. For instance, in Nigeria, an average person consumes in diet only eight

grams of animal protein per day which is less than the minimum requirement of 65g per adult per day recommended by the National Research Council (FAO, 2014). To overcome the problem, animal producing industries are making efforts by researches to increase population of livestock especially in the poultry sector with short generation interval in production. In addition, poultry are highly prolific, good feed converter (Smith, 2001) and the production involves the least hazardous and arduous process relative to other farmed animals (Oluyemi and Roberts, 2007). However, achieving maximum performance and cost return benefits in poultry production depend on quality and quantity of feeds. The quantity of feed requirement for intensive livestock production accounts for more than 75% of the total cost of production especially the production of monogastric animals like poultry that share the same staple foodstuffs with humans. To reduce production cost, increase novel micro livestock and animal protein in diets of the common Nigerian, alternative sourcing to the scare conventional foodstuff necessitated research on Japanese quails using castor bean residue or cake. Japanese quail (*Corturnix corturnix japonica*) is a small-sized bird with early maturing and prolific rate in addition to its unique traits and merits such as early attainment of sexual maturity, short generation interval, attainment of market weight of 150-180 g within 5-6 weeks of age and a high rate of egg production between 180-250 eggs per year (Shwarts and Allen, 1981; Garwood and Diehl, 1987). Furthermore, quails require less floor space, 8 to 10 adult quails can be housed in a space meant for just one adult chicken (Haruna *et al*. 1997). Quails require 20-25 g less feed per day, their meat and egg are high in protein quality with low cholesterol content and their meat is tender, tasty and highly acceptable making the meat a choice protein for high blood pressure persons as well as their eggs (Haruna *et al.* 1997). Japanese quails also can be produced with small capital and short day length period.

On the other hand, inadequacy and escalating cast of traditional feeding stuffs in Nigeria are what prompted research on alternative and use of castor seed residue after oil extraction.

The defatted seed cake has high protein content and other nutrients warranting it a potential rich protein source for food animals. Despite the relative abundance of *Ricinus communis* and its high nutrients content, use of the residue in diet for domestic animals is hindered by toxic phytochemicals namely ricin (1.5 w/w defatted), the most lethal of toxins (Audi *et al.* 2005). The other phytochemicals are ricinine-an agglutinin, allergen CB-IA, cyanide, phytic acid, and lipase. Attempts to detoxify castor residues for use in animal nutrition are usually aimed at removing of ricin, ricinine and allergens in the raw beans (Audi *et al.* 2005; Ani and Okorie, 2005). This investigation therefore attempted the use of solid state fermentation with *Aspergillus niger* as a bio-degrader in addition of calcium oxide to potentiate detoxification of castor seed meal in nutrition of *Corturnix corturnix japonica*.

MATERIALS AND METHODS

Castor seeds obtained from castor plants grown on Ilorin soil, Ilorin, Kwara State of Nigeria, were de-hulled using a de-hulling machine and ground in a hammer mill into paste. The paste was de-fatted by mechanical hydraulic oil press and residue (cake) subjected to solid state fermentation with the spores of *Aspergillus niger*. *Aspergillus niger* spores were generated by suspension in yeast extract broth at a concentration of 9×10^6 spores/mL (Pandey and Larroche, 2008). After the inoculation, 20 kg of the cake meal mixture was placed in a 100-litre bowl and covered with a muslin cloth and left to incubate for 7-days at room temperature (27 ˚C). The one week period was for the *A. niger* to effect degradation of the castor bean cake anti-nutritional factors. Incubation was terminated on the seventh day by oven-drying the solid state fermented stuff at 100 ˚C. 40g/kg CaO was added to the fermented cake meal for use in the diet mixtures. Removal or detoxification of castor bean anti-nutrients is easier when carried out in an alkaline environment (Pandey and Larroche, 2008).

One hundred and twenty Japanese quail chicks averaging 7.40g initial weight/chick at day old hatched from eggs incubated in the Departmental hatcher (incubator) were used for the experiment. The day old birds were first fed a commercial broiler starter mash for one week for adaptation. After the 7-days acclimatization, they were weighed, wing branded and randomly allotted to the four dietary treatments in triplicate lots of 10 chicks per replicate in a single factor design experiment. The diets contained 0.00, 2.50, 5.00 and 7.50% of the TCSM at the expense of the conventional protein source, soybeans, corresponding to diets 1, 2, 3 and 4, respectively. The control diet contained maize and soybean meals as basic ingredients. Birds were fed *ad libitum* during a feeding trial that lasted 56 days (8 weeks). Use of the quail in this trial followed the Ethical Protocols of the Committee of Animals Use of the University of Ilorin and that of Global Standard Practices. The composition of the experimental diets on as fed basis and its analysed nutrient contents of the diets is presented in Table 2.

Data collection / response criteria

Data on the nutritional composition of native milled seed, defatted and treated castor seed meals (TCSM) collected were subjected to descriptive statistics.

Table 1 Nutritional and phytochemical composition of milled raw seed, defatted and treated seed residue, cake

Parameters (%)	Raw seed	Defatted CSM	Treated CSM
Dry matter	94.70	99.24	89.14
Crude protein	36.40	35.66	35.09
Ether extract	51.19	36.87	22.09
Total Ash	4.28	7.04	11.67
Crude fibre	0.89	5.67	3.54
Soluble carbohydrate	8.44	14.76	47.61
Gross energy (kcal/g)	7.26	6.36	6.24
Phytochemicals (%)			
(Anti-nutrients)	Raw	Defatted cake	Treated
Ricin	0.10	0.070	0.065
Allergenic protein	0.30	0.240	0.230
Ricinine	0.10	0.048	0.046

CSM: castor seed meal.

Table 2 Composition of the experimental diets with analyzed nutrient contents (kg/100 kg diet)

Diets (%)	1	2	3	4
Treated castor seed meal	0.00	2.50	5.00	7.50
Maize	52.60	48.60	48.00	47.60
Soybean meal	25.00	22.50	21.00	18.00
Fish meal	4.00	5.00	6.00	6.50
Wheat offal	15.00	18.00	17.00	17.00
Dicalcium phosphate	2.00	2.00	2.00	2.00
Oyster shell	0.80	0.80	0.40	0.80
Salt	0.25	0.25	0.25	0.25
Vitamin and mineral premix[1]	0.20	0.20	0.20	0.20
Methionine	0.15	0.15	0.15	0.15
Total (%)	100	100	100	100
Analysed diet nutrient content (%)				
Dry matter	90.33	89.31	90.93	89.50
Crude protein	20.17	21.94	22.94	21.25
Ether extract	4.73	4.97	5.23	3.12
Ash	5.55	12.94	12.97	13.10
Crude fibre	4.29	6.81	10.50	11.61
Nitrogen free extract (NFE)	65.26	53.34	48.36	50.92
Gross energy (GE) (kcal/g)	4.53	4.26	4.25	4.15

[1] Premix supplied: vitamin A: 200000 1U; vitamin D_3: 400000 1U; vitamin E: 8.00 g; vitamin K: 0.40 g; vitamin B_{12}: 0.32 g; vitamin B_2: 0.96 g; vitamin B_6: 0.56 g; vitamin C: 2400 mg; Folic acid: 0.16; Biotin: 8.00 mg; Choline: 48.00 g; Calcium panthothiolate: 1.6 g; Manganese: 16.00 mg; Fe: 8.00 mg; Zinc: 7.20 g; Copper: 0.32 mg; Iodine: 0.25 mg; Cobalt: 36.00 mg; Selenium: 16.00 mg and butylated hydroxy toluene (BHT): 32.00 g.

In the course of the feeding trial, data were collected on daily feed consumption, weekly body weight gain while growth was calculated. Mortality rates (number of dead birds per replicate/diet) and efficiency of feed utilization (F/G) were also computed. A week before termination of the experiment, nutrients digestibility trials were conducted for a 72 h period and fecal samples collected from each replicate of a treatment containing feces as well as urinary paste were properly air-dried and analyzed for the determination of apparent nutrients retention. Percent of nutrients retention was calculated using the formula (Van Soest, 1982):

((Nutrient in feed-Nutrient in faeces)/(Nutrient in feed)) × 100

Data for haematological and serum biochemistry

Blood collection made from the external jugular vein of the quail for the determination of haematological and serum biochemistry was conducted in the morning of the following day (between 8-9 a.m.). Blood samples for haematological indices were collected in heparinized (EDTA) sample bottles while samples for biochemical parameters were taken in bottles without the anticoagulant, allowed to clot at room temperature before centrifuging at 3000 rpm to obtain clear sera. The sera were stored at -20 ˚C for the analysis of biochemical determinants.

Chemical analyses

Proximate and quantification of CSM anti-nutrients in the raw, defatted and treated cake meal, and proximate composition of diets and fecal samples were carried out by the methods of AOAC (2000). Haematological parameters were determined with the auto-haemocytometer while mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) values were calculated using the formula descried by Schalm *et al*. (1975).

Table 3 Dietary effect of treated CSM on performance of quail

Diets	1	2	3	4	SEM
Treated castor seed meal inclusion (%)	0.00	2.50	5.00	7.50	
Feed intake (g/bird/day)	16.74^{c}	11.23^{b}	9.38^{a}	7.10^{a}	0.29*
Weight gain (g/bird/day)	18.39^{c}	12.16^{ab}	9.95^{a}	8.94^{a}	0.31*
Growth rate	84.66^{c}	77.27^{b}	74.35^{b}	68.10^{a}	0.82*
Feed efficiency (F/G)	0.91	0.92	0.94	0.78	0.01^{ns}
Mortality (birds/diet)	0	5	7	10	

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
SEM: standard error of the means.
NS: non significant.

Table 4 Nutrients retention in quail fed processed dietary castor seed meal (CSM)

Diets	1	2	3	4	SEM
Treated castor seed meal inclusion (%)	0.00	2.50	5.00	7.50	
Dry matter intake	67.82	73.32	69.00	71.21	0.10^{ns}
Crude protein	48.10	60.30	55.41	62.17	0.69^{ns}
Crude fat	78.59	89.35	89.78	82.02	0.51^{ns}
Crude fibre	71.33	74.47	94.10	72.68	1.98^{ns}
Soluble carbohydrate	279.60^{a}	163.73^{b}	128.03^{b}	98.87^{b}	0.002*

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
SEM: standard error of the means.
NS: non significant.

Analysis of the biochemical indices was conducted using the clinical chemistry semi-auto-analyzer and a commercial biochemical assay kit. Enzyme activities of aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were analyzed by the spectrophotometric linked reaction methods (Reichling and Kaplan, 1988). Serum electrolytes were determined by the method of Young (2001).

Statistical analysis

Data on proximate composition and quantification of CSM anti-nutrients in the Raw, de-oiled and treated cake meal was subjected to descriptive statistics while data on performance, haematological and serum biochemistry were analysed by analysis of variance (ANOVA) following the design of a one-way classification. Significant differences between treatments means were separated by Duncan multiple range test as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Result on proximate analysis of castor seed (Table 1) shows that the seed contains valuable nutrients such as dry matter, crude protein, crude fat, crude fibre, soluble carbohydrate (NFE). These nutrients could be made available to the fed animals for utilization when the anti-nutrients present in the unprocessed seed are removed or reduced to minimum threshold. Treating the milled raw seed by solid state fermentation with *Aspergillus niger* microbes as bio-degrader and addition of calcium oxide (CaO) to potentiate detoxification of the castor seed cake meal (Table 1) resulted in reductions of the castor phytotoxins, ricin, ricinine and the allergens phytochemicals determined to give low levels of 0.065, 0.046 and 0.23, respectively, relative to the values on the native or raw milled seed. Detoxification of castor seed anti-nutrients is better made in alkaline medium. Dietary effects of treated CSM on performance of quail are shown in Table 3. Castor seed meal treatments reduced feed consumption, body weight gain, growth and increased casualties (mortality rate) in the course of the four weeks feeding experiment. Decreases in feed intake, weight gain, growth rate and survival rate responded correspondingly as the inclusion level of the seed meal increased in diets (P<0.05), suggesting, but not proving the residual toxic effects of the castor seed meal subjected to fermentation/CaO treatment since processing of the castor seed was done only in one batch. Corrections for birds being removed (by death) within the interval of the feeding trial were made by calculations. Retention of nutrients in quails (Table 4) however, was not influenced by castor seed meal inclusions in diets hence results on the test feedstuff diets and that of the control diet was comparable (P>0.05).An exception to this finding was retention of soluble carbohydrate (NFE) which decreased concomitantly with increasing dietary levels of castor seed meal in comparison with the reference diet (P<0.05). Data on haematological parameters (Table 5) indicated that treating CSM by the methods adopted caused variations in values obtained on haematopoiesis. Most of the indices on blood composition (red blood cell (RBC), hemoglobin (Hb), platelet, white blood cell (WBC), with its differential counts as well as MCV, MCHC) appeared comparable with those on the control diet (P>0.05) but PCV and MCH values presented increases with increasing dietary levels of TCSM relative to the reference diet (P<0.05).

Table 5 Influence of processed castor seed meal (CSM) on blood composition in quail

Diets	1	2	3	4	SEM
Treated castor seed meal inclusion (%)	0.00	2.50	5.00	7.50	
Red blood cell (RBC) ($\times10^{12}$/L)	2.00	2.55	2.53	2.10	0.14^{ns}
Hemoglobin (Hb) (g/dL)	6.93	7.70	10.70	9.90	5.58^{ns}
Packed cell volume (PCV) (%)	0.73^{a}	1.00^{b}	1.10^{b}	1.10^{b}	0.34*
Mean corpuscular volume (MCV) (Pg)	131.67	161.67	177.67	154.67	1.00^{ns}
Mean corpuscular haemoglobin (MCH) (Pg)	33.77^{a}	44.00^{b}	42.63^{b}	43.70^{b}	3.75*
Mean corpuscular haemoglobin concentration (MCHC) (%)	26.27	27.63	24.20	28.00	5.19^{ns}
Platelet ($\times10^{9}$/L)	353.67	447.00	308.33	222.67	0.70^{ns}
White blood cell (WBC) ($\times10^{9}$/L)	10.76	9.74	9.10	7.787.78	2.69^{ns}
White blood cell (WBC) differential counts					
Monocytes (%)	1.00	0.67	2.00	2.00	0.80^{ns}
Eosinophils (%)	0.33	0.00	0.33	0.00	0.37^{ns}

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
SEM: standard error of the means.
NS: non significant.

Table 6 Biochemical Indices in quail fed dietary treated castor seed meal (CSM)

Diets	1	2	3	4	SEM
Treated castor seed meal inclusion (%)	0.00	2.50	5.00	7.50	
Blood glucose (mmol/L)	9.70^{a}	9.50^{a}	12.96^{b}	19.00^{c}	3.64*
Serum protein (g/L)	62.33	48.33	59.00	56.67	0.62^{ns}
Serum albumin (g/L)	29.33	30.33	27.33	30.67	3.65^{ns}
Serum globulin (g/L)	16.00	15.00	23.00	16.00	0.24^{ns}
Albumin/globulin ratio	1.98	2.11	1.37	1.97	0.18^{ns}
Aspartate amino transferase (AST) (IU/L)	180^{d}	175^{c}	94^{b}	79.66^{a}	4.82*
Alanine aminotransferase (ALT) (IU/L)	89.37^{b}	102.26^{c}	109.10^{d}	65.56^{a}	6.34*
Alkaline phosphatase (ALP) (IU/L)	1424	1716	1749	2271	4.27^{ns}

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
SEM: standard error of the means.
NS: non significant.

Table 7 Dietary impact on blood electrolytes in quail given processed castor seed meal (CSM)

Diets	1	2	3	4	SEM
Treated castor seed meal inclusion (%)	0.00	2.50	5.00	7.50	
Ca^{2+} (mmol/L)	2.70^{c}	2.00^{c}	1.54^{b}	0.99^{a}	0.92*
Na^{+} (mmol/L)	139	132	141	134	1.12^{ns}
K^{+} (mmol/L)	5.63^{b}	6.33^{b}	4.17^{a}	3.90^{a}	2.37*
Cl- (mmol/L)	53.00	71.00	72.00	45.00	3.02^{ns}
Mg^{2+} (mmol/L)	0.73	1.00	1.07	1.10	0.15^{ns}
HCO_3^{-} (mmol/L)	27.67^{c}	26.33^{c}	21.67^{b}	17.67^{a}	0.35*

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
SEM: standard error of the means.
NS: non significant.

Deviation in these values from the control value may be inimical to the wellbeing of the birds.

Biochemical determinants in quails (Table 6) receiving dietary treated CSM indicated increment in blood glucose level (P<0.05) compared to the conventional diet and values on serum total protein, albumin, globulin, albumin/globulin ration were not different from those on the control diet (P>0.05). Feeding the treated seed meal caused decreases in the activities of the transaminases (AST and ALT) especially at the highest inclusion of 7.50% CSM (P<0.05). Reduction in activity of the enzymes may reduce the functions they are supposed to perform in the animal body.

Profiling electrolytes in the quails given dietary treated CSM (Table 7) revealed that treatment resulted in changes in mineral constituent in the birds. Treatment failed to improve availability and utilization of calcium, potassium and bicarbonate electrolytes (Ca^{2+}, K^{+} and HCO_3^{-}) as values on these electrolytes decreased in response to increasing level of CSM in diets (P<0.05).

CONCLUSION

This experiment established that in spite of treatment by solid state fermentation with addition of CaO, residual toxic effect of castor seed meal prevailed to elicit adverse effects

on the fed animals. Further works to detoxify and determine acceptable levels of inclusion of the castor seed in diets of food animals are in progress.

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REFERENCES

Ani A.O. and Okorie A.U. (2005). The effect of graded levels of dehulled and cooked Castor oil bean (*Ricinus communis*) meal on performance of broiler starters. *Nigerian J. Anim. Prod.* **32(1),** 54-60.

AOAC. (2000). Official Methods of Analysis. Vol. I. 18th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Audi J., Belson M., Patel M., Schier J. and Osterloh J. (2005). Ricin poisoning : a comprehensive review. *J. Am. Med. Assoc*. **294(18),** 2342-2351.

FAO. (2014). Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Garwood A.A. and Diehl R.C.J. (1987). Body volume and density of live *Coturnix* quail and associated genetic relationships. *Poult. Sci.* **66 (8),** 1269-1272

Haruna E.S., Musa U., Okewole P.A., Shemaki D., lombin L.H., Molokwu J.U., edache J.A. and Kaarsin P.D. (1997). Protein requirement of quail chicks in plateau state, Nigeria. *Nigerian Vet. J*. **18,** 108-113.

Oluyemi A.J. and Roberts F.A. (2007). Poultry Production in Warm West Climates. Spectrum Books Ltd., Ibadan. Nigeria.

Pandey A. and Larroche C. (2008). Current Development in Solid State Fermentation. Published by Springer, Germany.

Reichling J.J. and Kaplan M.M. (1988). Clinical use of serum enzymes in liver diseases. *Digest. Dis. Sci*. **33,** 1601-1614.

Schalm O.W., Jain N.C. and Carroll E.J. (1975). Haematology. Publisher: Lea and Febiger, Philadelphia, US.

Smith A.J. (2001). Poultry. Macmillan Publishers Ltd., London, United Kingdom.

Steel R.G.D. and Torrie J.H. (1980). Principles and Procedures of statistics, a Biometrical Approach. Publisher: McGraw-Hill Companies, New York.

Van Soest P.J. (1982). Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, New York.

Young D.S. (2001). Effect of Diseases on clinical Laboratory Tests. American Association for Clinical Chemistry Press, Washington, D.C.