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Total Protein Concentrations in Brain Regions and Testicular/Tubal Fluids of Rabbits Fed Cassava Peel Meal as Replacement for Maize

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Abstract

Cheaper unconventional feed materials whose metabolizable energy is similar or closed to maize could be used in animal diets. This study investigated the effect of replacing maize with cassava peel meal (CPM) on the total protein concentrations in the brain regions and testicular/tubal fluids of rabbits. Sixty crossed bred weaned rabbits with an average initial bodyweight of 559.58 ± 0.42g/rabbit were used. The animals were assigned to experimental diets using a completely randomised design. Five experimental diets were formulated with ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 maize: sun dried CPM for T_1, T_2, T_3, T_4 and T_5, respectively. At the end of the feeding trial, six rabbits per treatment were sacrificed by stunning and decapitation. The heads were severed and immediately dissected for brain harvest. The various brain regions were separately homogenized. The testes and epididymis of the bucks as well as segments of the female reproductive tract were also homogenised and total protein concentrations determined by the biuret method. Results showed that the total protein concentrations in all the brain regions were significantly ($P < 0.05$) affected by dietary treatments except the medulla oblongata. The protein concentration decreased as the level of CPM replacement increases with 100% level recording the least values. The protein concentration in the testicular/tubal fluids recorded a fluctuating trend that could not be ascribed to diets. The study concluded that cassava peel meal could serve as a better substitute for maize up to 75% without any serious adverse effects on the brain and reproductive organs of rabbits.

Keywords: *Brain; Cassava Peel; Protein; Rabbits; Reproduction*

Introduction

The problem of low protein intake has persisted in most third world countries for quite a long time now. Nigeria is among these countries with almost 70% of the populace being rural dwellers and low income earners [1]. It becomes very difficult for these classes of people to meet the required minimum daily protein intake of 35g per caput per day set by the Food and Agriculture Organization [2]. The problem is complicated by the geometric growth in human population and the prevailing feed crisis. The resultant effect of this increasing human population is the gross inadequate supply of grains due to competition for the use of the products for food and feed respectively. Furthermore, it also intensifies the problems of malnutrition [3]. Relying on the traditional livestock species like cattle, sheep and goat will continue to intensify the problem of low protein intake among the populace, due to their long reproductive cycle. There is therefore

the need to source for an inexpensive animal protein source, which could become a solution to successful improvement of the nutritional status and health of many poor people especially children [4].

The domestic rabbit (*Oryctolagus cuniculus*) is a medium sized hopping animal with long ears and short tail [5]. Best known for being prolific, the rabbit is able to solve the protein and nutritional needs of the poor populace. The qualitative and profitable attributes of the rabbit over other livestock species cannot be overemphasized; these include small body size, short generation interval (30 ± 2 days [4]; rapid growth rate, less land is required for rabbit production, early sexual maturity and ability to rebreed shortly after parturition. Rabbits have the capacity to convert feed of very low quality to meat [6].

According to FAO [7], animal feeds are the pivot for food industry globally. Therefore, for profit maximization; rabbit production in Nigeria and other developing countries should be geared towards exploring input resources which can reduce cost of feeding which account for 70% of the total cost of production to the minimum and can also moderately improve growth rate and reproductive performance without negatively affecting carcass characteristics [8]. Good and cheap animal feeds make livestock production more viable throughout the world. A major setback in the livestock industry is the competition of the cereal/grain based feed stuff [9]. The potentials of some agricultural and industrial waste materials that can be moderately processed and incorporated in livestock feeds could therefore be exploited. According to Ojebiyi., *et al.* [6] availability is one of the major factors determining the suitability of any non - conventional feedstuff to avoid scarcity that may eventually increase the price.

Cassava (*Manihot esculenta* crantz) is an all season crop, widely cultivated in the tropical region and ranks among the top 10 crops. Cassava thrives well where other crops fail. It is estimated that almost 65 percent of the cassava crop is used for human consumption and the remainder used for animal feed and other industrial purposes [10]. Cassava peels are by- products of cassava. They constitute wastes in the cassava industry and accounts for 5 - 15 percent tuber by weight [11]. The peels are derived after the tubers have been peeled mechanically. The cassava peels contain a higher level of protein than other parts. Cassava peels have the following composition: 27.9% dry matter, 5.3% crude protein, 5.93% ash, 66.6% nitrogen free extracts and 1.2% ether extract. It is relatively high in crude fibre (20.97%) [12].

The only limiting factor in the use of cassava and its by-products is the presence of hydrocyanic acid (HCN), which is detrimental especially to monogastrics. The main toxic principle which is found in varying degree in every part of the cassava plant is a chemical compound called linamarin. It is a cyanogenic glucoside which is converted to toxic hydrocyanic acid. Hydrocyanic acid is a hydrophilic compound, thus can readily dissolves in water. Cyanide is more abundant in the cassava peels than other parts of the product. Long term consumption of small amount of cyanide can cause severe health problems such as tropical neuropathy [12].

In view of the health and economic benefits of rabbit production as well as the availability of cassava peels in this ecozone, this study was therefore designed to determine the effect of replacing maize with cassava peel meal on the total protein concentrations in some brain regions and testicular/tubal fluids in rabbits.

Materials and Methods

Location of the study

The study was carried out at the Rabbitry Unit of the Teaching and Research Farm, University of Calabar, Calabar, Cross River State, Nigeria. According to the GeoNames geographical database by Google earth [13]; Calabar is located at 4.9517^0 latitude of the equator and 8.322^0 longitude of the Greenwich meridian (all in decimal degrees) with an average elevation/altitude of 42 metres above sea level. The annual rainfall ranges from 3000 - 3500 mm and the average daily temperature is $25^0C/77^0F$ and increase to 30^0C (86^0F) in August. The relative humidity is between 70 and 80% while the wind speed/direction is 8.1 km/h west and the cloud is broken at 1000ft with little cumulonimbus at 2200ft.

Processing of the test ingredient (Cassava peel meal, CPM)

Fresh composite cassava peels were collected from cassava processing locations in Odukpani and Akamkpa LGAs of Cross River State, washed and sun dried for seven to eight days until constant weight was achieved. The peels thereafter milled using a hammer mill. The milled samples were stored in sterile polythene bags at room temperature 23^{0}C and kept to be used as replacement for maize in the test diets. Cassava peel meal served as the test ingredient while the major feed ingredients were maize, full fat soybean, rice husk, palm kernel meal, and crayfish dust. The proximate composition of sun dried cassava peel meal was determined using the A.O.A.C [14] methods.

Experimental diets

Five experimental diets were formulated each comprising ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 maize: sun dried CPM for T_1, T_2, T_3, T_4 and T_5 respectively. Treatment (T_1) served as the control diet. The proximate composition of the experimental diets was done based on the procedures outline by AOAC [14]. The gross composition of experimental diets is presented in table 1.

Ingredient	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)
Yellow maize	30.00	22.00	15.00	7.50	0.00
Soybean meal	12.70	15.60	17.60	18.60	19.79
Cray fish dust	4.00	4.00	4.00	4.00	4.00
PKC	7.00	11.10	12.10	12.80	12.91
Wheat offal	15.00	10.00	9.00	9.20	7.00
Rice husk	25.00	23.00	21.00	19.10	20.00
Cassava peel meal	0.00	7.50	15.00	22.50	30.00
1Bone meal	2.50	2.50	2.50	2.50	2.50
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Premix	0.20	0.20	0.20	0.20	0.20
Salt	0.10	0.10	0.10	0.10	010
Palm oil	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100	100	100
Calculated nutrients					
% CP	16.21	16.95	17.17	17.43	17.33
% CF	9.76	10.05	10.31	10.61	10.47
ME (Kcal/kg)	2,482.05	2,391.53	2,279.28	2,155.90	2,022.02

Table 1: *Gross composition of experimental diets.*

Gross Composition of Bio-Super Premix per Kg; Vitamin A-1,500,000 IU; Vitamin D3-300,000 IU; Vitamin E-400 mg; Vitamin B2-400 mg; Vitamin B12-2,000 mg; Nicotinamide-2,000 mg; Calcium D Panto thenate-800 mg; Choline Chloride-40,000 mg; Ferrous Sulphate-2,000 mg; Manganate Sulphate-5,000 mg; Copper Sulphate-80 mg; Zinc Oxide-3,000 mg; Cobalt sulphate-10 mg; Potassium Iodide-120 mg; DL-Methionine-10,000 mg and Antioxidant-18,000 mg. The premix was manufactured by Bio-Phamachemic Company HCM city, CF: Crude Fibre; CP: Crude Protein; SCPM: Sun Dried Cassava Peel Meal; SBM: Soybean Meal; PKC: Palm Kernel Cake; ME: Metabolizable Energy.

Experimental animals and management

A total of 60 (30 does and 30 bucks) crossed bred weaned rabbits aged between 5 and 6 weeks old with an average initial weight of 559.58 ± 0.42g were used in this study. The rabbits were sourced from a standard rabbitry in Calabar metropolis. The animals were

housed in wooden double tier cages, covered with wire mesh and measuring 65 × 65 × 65 cm and raised 25 cm from ground level. The animals were assigned to experimental diets using a completely randomised design (CRD). The cages and entire rabbitry were thoroughly washed with a strong disinfectant and allowed for seven days to dry before animals were brought in. A concrete watering trough and a fabricated feeding trough were placed in each hutch. Immediately on arrival the rabbits were given anti-stress vitalyte via drinking water. The rabbits were then randomly distributed into five different treatment groups after equalizing for weight, with a stocking ratio of 4 bucks: 4 does (i.e. 8 rabbits per treatment). Two rabbits each (of same sex) occupied a cage to make up a replicate. The rabbits were screened against ecto and endo parasites using Ivomec. The rabbits were fed the control diet plus a small uniform quantity (40g) of supplementary forage (*Tridax procumbens*) within the two weeks of rabbits' acclimatization to the rabbitry environment. The actual feeding trial proceeded thereafter for nine weeks duration and water was provided *ad libitum*.

Determination of total protein concentrations in brain regions of rabbits fed cassava peel meal based diets

At the end of the feeding trial, six rabbits per treatment (3 bucks and 3 does) were starved for 12 hours and thereafter sacrificed by stunning and decapitation. The head were severed at the occipito-atlantal joint and immediately placed in ice containers for easy identification. All the heads were then frozen at -20^{0}C for two weeks before dissection as recommended by Bitto., *et al* [15]. During dissection, each head was placed in a dorsoventral position on ice - cold porcelain tile for cutting before the brain was carefully dug out by blunt dissection. The brain was then freed of adhering meninges, weighed and dissected out as described by Egbunike [16]. Samples of each brain region (cerebral cortex, cerebellum, amygdala, hypothalamus, pons, mesencephalon, hippocampus and medulla oblongata) were then weighed using a sensitive digital balance (Kerro model) and separately homogenized in 1% (w/v) 0.1 M ice - cold phosphate buffer containing 0.10% Triton X- 100 (sigma- Aldrich St Louis, USA). The total protein concentrations in the brain region were evaluated by the Biuret method as outlined in the Boehringer Mannheim Diagnostica manual as reported by Gbore and Egbunike [17].

Determination of total protein concentration in the testicular/epididymal/tubal fluids of rabbits

The testes and epididymes of the bucks as well as segments of the female reproductive tract (ovaries, oviducts, uterine horns, cervices and vagina) were flushed with 0.15M normal saline as reported by Egbunike and Adegunle [18]. Thereafter, the total protein concentrations of the fluids from testes, epididymes (caput, corpus and cauda) and segments of the female tract were determined by the Biuret method as outlined in the Boehringer Mannheim Diagnostica manual as reported by Gbore and Egbunike [17].

Statistical analysis

All data obtained in this study were subjected to one - way analysis of variance (ANOVA) for CRD. Significant means were separated using the New Duncan Multiple Range Test [19].

Results

Weight of brain and total protein concentrations in brain region of rabbits

The result of the weight of brain and total protein concentrations in the brain regions of rabbits of rabbits fed diets containing cassava peel meal is presented in table 2. The weight of the brain was significantly ($P < 0.05$) different between treatments. Treatments 1 (0%) and 2 (25%) were significantly ($P < 0.05$) higher than treatments 3 (50%), 4 (75%) and 5 (100%) which were statistically similar. The increase in the level of replacement of CPM followed a decreasing trend in the weight of the brain. The values obtained in the study were 9.45, 9.25, 7.04, 6.56 and 6.50g for treatments 1, 2, 3, 4 and 5 respectively. The total protein concentrations in the cerebellum was significantly ($P < 0.05$) affected by the dietary treatments. Treatments 1 and 2 showed higher protein concentrations than treatments 3, 4 and 5 that were statistically similar. The results were 1.04, 1.15, 0.58, 052 and 0.46 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively. The hypothalamus showed a significant ($P < 0.05$) difference in the amount of total protein concentration in the dietary treatments. Treatment 3 was significantly ($P < 0.05$) higher while treatment 4 recorded the least level of protein concentration. The values obtained in the study were 0.65, 0.52, 1.34, 0.28 and 0.88 g/100 ml respectively. The amount of protein concentration in the cerebral cortex was significantly

(P < 0.05) affected by the dietary treatments with treatments 2, 3 and 4 being significantly higher than treatment 1 and 5. The results obtained were 0.40, 0.68, 0.64, 0.69 and 0.35 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively. The pons showed significant (P < 0.05) difference between the dietary treatments. Treatment 3 was significantly higher 1, 2 and 4, while treatment 5 showed the least level of increase in protein. The result for treatments 1, 2, 3, 4 and 5 were 0.44, 0.64, 0.81, 0.26 and 0.22 g/100 ml respectively. The total protein concentration of the mesencephalon was significantly (P < 0.05) different between the dietary treatments. Treatments 2, 3, 4 and 5 were significantly higher than treatment 1 which recorded the least level of total protein concentration. The values obtained in the study were 0.39, 0.70, 0.68, 0.70 and 0.78 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively. The total protein concentration in the amygdala was significantly (P < 0.05) affected by the dietary treatment. Treatment 1 recorded the least value (total protein concentration) while treatment 4 was significantly higher among dietary treatments. The values obtained in the study were 0.27, 0.40, 0.55, 0.99 and 0.38 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively. The medulla oblongata was not significantly (P > 0.05) influenced by the varying dietary levels of CPM. The results obtained were 0.99, 1.42, 1.33, 0.75 and 1.34 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively.

Parameter	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)	S.E.M
Weight of brain	9.45^{a}	9.25^{a}	7.04^{ab}	6.56^{b}	6.50^{b}	0.37
Cerebellum	1.04^{a}	1.15^{a}	0.58^{b}	0.52^{b}	0.46^{b}	0.05
Hypothalamus	0.65^{c}	0.52^{c}	1.34^{a}	0.28^{d}	0.88^{b}	0.07
Cerebral cortex	0.40^{b}	0.68^{a}	0.64^{a}	0.69^{a}	0.35^{b}	0.02
Pons	0.44^{b}	0.64^{b}	0.81^{a}	0.26^{d}	0.22^{d}	0.04
Mesencephalon	0.39^{b}	0.70^{a}	0.68^{c}	0.70^{a}	0.78^{a}	0.02
Amygdala	0.27^{bc}	0.40^{ab}	0.55^{c}	0.99^{a}	0.38^{ab}	0.04
Medulla oblongata	0.99	1.42	1.33	0.75	1.34	0.13

Table 2: *Weight of brain (g) and total protein concentrations (g/100 ml) in brain regions of rabbits fed cassava peel meal as replacement for maize.*

^{abc}: Means on the same row with different superscript are significantly (P < 0.05) different.

S.E.M: Standard Error Mean.

Total protein concentrations in testicular and tubal fluids of rabbits

Results showing the total protein concentration in testicular and tubal fluids of rabbits fed cassava peel meal as replacement for maize are presented in table 3. The total protein concentration in the testes showed a significant (P < 0.05) difference between the dietary treatments. There was a steady increase as the level of CPM replacement increased. Treatment 5 had the highest total protein concentration level while 1 had the least. The remaining treatments, 2, 3 and 4 were statistically similar. The results obtained were 1.28, 1.64, 1.83, 1.76 and 2.16 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively. The level of total protein concentration in the paired caput epididymis was significantly (P < 0.05) affected by the dietary treatments. The values obtained from the study were 0.58, 1.25, 1.32, 1.06 and 2.09 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively. The total protein concentration in the paired corpus epididymis was significantly (P< 0.05) influenced from the dietary treatments. Treatments 2 and 3 were significantly higher than treatment 5, while treatments 1 and 4 where statistically the same. The values obtained in the study were 0.85, 1.08, 1.07, 0.08 and 0.04 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively. The total protein concentration of the paired cauda epididymis was significantly (P < 0.05) affected by the dietary treatments. The results obtained were 0.73, 0.81, 0.79, 1.05 and 0.80 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively. The total protein concentration in the ovary was significantly (P < 0.05) affected by the treatment. The level of total protein concentration increased with the increasing levels of CPM replacement in the diets. The results obtained in the study were 0.60, 0.73, 0.86, 1.00 and 1.15 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively.

Parameter	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)	S.E.M
Bucks						
Testes	1.28^{c}	1.64^{b}	1.83^{ab}	1.76^{b}	2.16^{a}	0.08
Paired caput	0.58^{d}	1.25^{bc}	1.32^{b}	1.06^{c}	2.09^{a}	0.13
Paired corpus	0.82^{b}	1.08^{a}	1.07^{a}	0.08^{b}	0.04^{c}	0.06
Paired cauda	0.68^{b}	0.95^{a}	0.59^{b}	0.32^{c}	0.28^{c}	0.06
Epididymis	0.73^{b}	0.81^{b}	0.79^{b}	1.05^{a}	0.80^{b}	0.06
Does						
Ovary	0.60^{e}	0.73^{d}	0.86^{c}	1.00^{b}	1.15^{a}	0.05
Oviduct	0.26^{c}	0.34^{c}	0.51^{b}	0.91^{a}	0.82^{a}	0.07
Uterine horns	0.70^{c}	0.81^{bc}	0.92^{b}	1.15^{a}	0.83^{bc}	0.04
Cervix	0.30^{c}	0.30^{c}	0.30^{c}	0.83^{a}	0.53^{b}	0.05
Vagina	0.27^{b}	0.32^{b}	0.25^{b}	0.46^{a}	0.32^{b}	0.02

Table 3: *Total protein concentrations (g/100 ml) in testicular and tubal fluids of rabbits fed cassava peel as replacement for maize.*

^{abc}: Means on the same roll with different superscript are significantly (P < 0.05) different.

S.E.M: Standard Error of Mean.

The total protein concentration in the oviduct showed a significant ($p < 0.05$) difference among the dietary treatments. Treatment 1 recorded the least level of total protein concentration. The values obtained in the study were 0.26, 0.34, 0.51, 0.91 and 0.82 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively. The result for the total protein concentration in the uterine horns showed significant ($P < 0.05$) differences between the dietary treatments. Treatment 4 was significantly higher than treatment 1 while treatment 2, 3 and 5 were similar. The results obtained were 0.70, 0.81, 0.92, 1.15 and 0.83 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively.

There was a significant ($P < 0.05$) difference between the total protein concentrations in the cervix for the different dietary treatments. The results of the total protein for the cervix were 0.30, 0.30, 0.30, 0.83 and 0.53 g/100ml for treatments 1, 2, 3, 4 and 5 respectively. The total protein concentration in the vagina was significantly ($P < 0.05$) affected by the dietary treatments. The results obtained were 0.27, 0.32, 0.25, 0.46 and 0.32 g/100 ml for treatments 1, 2, 3, 4 and 5 respectively.

Discussion

Weight of brain and total protein concentration in brain regions of rabbits fed cassava peel as replacement for maize

The weight of brain obtained in this study ranged between 6.50 and 9.45g. These values were lower than the value of 12g reported by Bitto., *et al* [15]. The difference could be attributed the variations in breed of rabbits and dietary treatments used in the separate studies. The total protein concentrations in the brain regions were significantly ($P < 0.05$) different between dietary treatments except the medulla oblongata. The cerebellum showed a significant ($P < 0.05$) decrease in the level of the total protein concentration with increasing level of CPM. Treatment 5 (100%) had the least level of protein concentration, suggesting a possible interference of residual cyanide in the CPM diets with neural mechanism involved in protein synthesis and rate of turnover in the cerebellum. The result corroborates the values of 0.47 - 1.14g recorded by (27) who also had a decrease in the level of protein concentration as the level of inclusion of pawpaw leaf diets increased [20]. The level of protein concentration in the hypothalamus decreased with increasing level of CPM concentration in the diets. In this study the values ranged between 0.40 and 0.65g. The obvious side effect of residual cyanide was clearly noticed in the hypothalamus. The function of the hypothalamus is to help maintain homeostasis (stability of the internal environment) in the body. The significant differences that were observed in all the regions of the brain segment except the medulla oblongata could be attributed to

changes in protein synthesis or metabolism arising from residual cyanide in cassava peel meal. Cyanide have been reported to be a protein inhibitor, which is capable of binding and interfering with enzymes and substrates that are needed in the activation, transcription and conversion process involve in protein synthesis. The residual cyanide could also interfere with proper brain development, affecting the function of protein in the brain, which is useful for the repair of worn-out tissues for growth, and development and also ensuring bioavailability of minerals through the binding process to some minerals. Protein in the brain is prone to changes especially during development, which are not attributed to genetic or species effect [16].

Total protein concentrations in testicular and tubal fluids of rabbits fed cassava peel meal as replacement for maize

The total protein concentration in the fluid from the testes ranged between 1.28 and 2.16 g/100 ml for all treatments. The trend in the testicular fluid showed a steady increase from treatment 1 to 5. The total protein concentration in the paired caput epididymis also recorded a steady increase across the treatments. However, the total protein in the corpus, cauda and epididymis showed a fluctuating trend across dietary treatments. Bitto [20] reported a lower value for paired testes, epididymis, paired caput, paired corpus and paired cauda for rabbit bucks fed kapok seed meal and forage combination. The total protein concentration in the tubal fluid of female rabbits ranged between 0.60 and 1.15 g/100 ml. As the level of CPM replacement increased, the protein concentration in the tubal fluid of the doe rabbits also increased. For ovary, oviduct and uterine horn, while the cervix and vagina showed a fluctuating trend. The values in this study were slightly higher than the report of Bitto and Egbunike [21] and Ozung., *et al.* [22] who fed paw-paw leaf meal and varying levels of CPM respectively to female rabbits; implying that residual cyanide might not have any adverse effect on the protein concentrations in the testicular and tubal fluids and by extension reproductive efficiency of rabbits.

Conclusion

This study concluded that the optimum level of cassava peel meal replacement for maize in the diets of rabbits is 75%, in view of its positive effect on the total protein concentrations in the brain regions as well as testicular and tubal fluids vis-à-vis reproductive potential of rabbits.

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