



Effect of Toasted *Jatropha curcas* (Physic Nut) Seed on the Semen Quality and Blood Characteristics of Male Albino Rats (*Rattus norvegicus*)

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Abstract

*The study was conducted to assess the effect of toasted *Jatropha Curcas* seed meal on semen quality and serum biochemical indices of albino rats (*Rattus norvegicus*). Sixty male albino rats were used in the study which lasted for the period of four weeks. Albino rats were allotted randomly into five (5) treatments and replicated three (3) times with four rats per replicate in a completely randomized design (CRD). Raw *Jatropha* seed were sundried for 15 days and subjected to toasting with the aim of reducing anti-nutritional factors available in the seeds. After toasting seeds were ground to reduce the particle size and used to compound the experimental diets. Five diets were compounded with toasted *Jatropha* seed meal replacing groundnut cake at 0.0, 2.5, 5.0, 7.5 and 10% replacement levels. The result of phytochemistry revealed the presence of some anti-nutritional factors which include tannins, saponins, phenols, alkaloids, glycosides and phytates which were significantly reduced in toasted *Jatropha* seeds. The biochemical parameters considered in the study (cholesterol, Urea and Creatinine) showed significant ($P < 0.05$) differences across the treatment groups but, they were normal ranges. Semen characteristics: semen pH and semen motility (active) differ ($P < 0.01$) significantly across the treatment groups. Similarly, morphology, sperm count and sperm density were highly ($P < 0.001$) significant and showed improvement when compared with the control. It could therefore be concluded that, semen quality may be improve leading to improvement in fertility when toasted *Jatropha* seed meal is incorporated in to the diet of albino rats without harmful effect on serum biochemistry indices.*

Key words: Albino rats, Toasted *Jatropha* seeds, Serum biochemistry and Semen quality

Introduction

Maintenance of breeder males for normal semen production is a substantial production cost and key to successful reproduction in the breeder segment of any endeavour. On a breeder farm, the most valuable traits are the semen characteristics which depend on the conditions of the male in the flock/herd. Various factors are keys in this respect. Rearing and managing them properly will have a positive influence on their fertility.

Jatropha has been a plant of interests to researchers over the years; because several recommendations were made for its use in formulating feed for animals (Makkar *et al.*, 2008; Belewu *et al.*, 2010; El-Zelaky *et al.*, 2011; Antyev, *et al* 2017). (Makkar *et al.*, 1998) reported that the protein content of *Jatropha curcas* seed meal has been

analysed, and it has shown to compete favourably with soybean meal containing a good balance of essential amino acids, with the exception of lysine. However, several researchers tried the use of *Jatropha* meal in different animals. Positive effects have been reported in fish (Makker and Becker, 1998; and Kumar *et al.*, 2010); goats, (Gadir *et al.*, 2003; and Katole *et al.*, 2013); Pigs (Chivandi *et al.*, 2006) and Sheep (Katole *et al.*, 2011).

Due to its (*Jatropha curcas*) abundance, unexploited and ready availability in this environment, the study was carried out to determine the effect of *Jatropha curcas* seed meal on semen quality and blood profile of albino rats.

Materials and Methods

Study Area

The study was conducted at rabbit unit of the Teaching and Research Farm of Modibbo Adama University, Yola, Adamawa State located within the Guinea Savanna Zone of Nigeria. The site is between latitude 9⁰ and 11¹ North and longitude 10⁰ and 12¹ East. The climatic is tropical with distinct dry and wet seasons. The rainfall starts in April and ends around late October, November to April marks the dry season. It has annual rainfall of about 700mm-1600mm and relative humidity that ranges from 5 to 42% and average maximum temperature of 39⁰C (Adebayo and Tukur 1999).

Sources of Albino Rats and *Jatropha curcas* seed

Male albino rats numbering sixty (60), weighing 150 ± 20 g were obtained from National Veterinary Research Institute (NVRI) Jos, Plateau State Nigeria, while the *Jatropha curcas* seeds were obtained from a local market in Daban Fulani, Kwami Local Government Area of Gombe State, Nigeria.

Processing *Jatropha curcas* seeds

The seeds were gathered and brought over in one container. The shell and coats were mechanically removed. The decorticated seeds were cleaned, dried under the sun for 15 days and then toasted by dried heat in a pan. The toasting was done at a temperature of 100⁰C with constant stirring for uniformity in heat and also to avoid burning. After the toasting, it was ground to reduce the particle size and incorporated in to the feeds to form the experimental diets. Table 1 shows the percentage compositions of the experimental diets.

Animals and their Management

On arrival, albino rats were housed in a specially designed propylene cages, and given standard grower diet (Vital Feeds) with water *ad libitum*. During the study, the experimental animals were given the control formulated diet without *Jatropha*, and were maintained in the laboratory under the ambient temperature of about (35⁰C) and 12 hours of light and dark cycle for seven days

to allow them to acclimatize before the commencement of the experiment. Guide for the care and use of laboratory animals 2011 of the Institute of Laboratory Animal Research (ILAR) division on earth and life studies, National Research Council (2011) was duly followed.

Proximate Analysis

The chemical analysis of the raw and toasted experimental diets were carried out to determine the dry matter (DM) content, crude protein (CP), crude fiber (CF), ether extract (EE), and Ash according to AOAC (2000) methods, while NFE and Metabolisable energy (ME) were calculated. NFE was calculated as $NFE = \%DM - (\%EE + \%CP) + \%Ash + \%CF$ and Metaboliserble energy was calculated according to the formula of Ponzenga (1985). $ME (Kcal/Kg) = 37 \times CP (\%) + 81.1 \times EE (\%) + 35.5 \times NFE (\%)$.

Experimental Diets and Design

Toasted *Jatropha* seed meal was incorporated at 0.0%, 25%, 5.0%, 75% and 10% levels replacing the GNC portion of the diets and designated as diets 1, 2, 3, 4 and 5 respectively. A total of sixty (60) albino rats were allotted to five dietary treatments and replicated three times with four rats per replicate in a completely randomized design (CRD). The rats were stabilized for a week, weighed and randomly allocated to their various treatment groups. The experiment lasted for a period of four weeks.

Semen and Blood collection and their Analyses

At the end of the study period semen samples from the experimental animals were collected from the epididymis. The rats were anesthetized using chloroform and the epididymis removed completely and placed into a container with a wide bore containing lactate ringer's solution to maintain osmolality and preserves the spermatozoa until examination. The specimen container is kept at ambient temperature between of about 37 °C, to avoid large changes in temperature that may affect the spermatozoa

after collection. The cauda epididymal was removed from each rat and the spermatozoa were squeezed in to a petri dish containing 5.0ml of physiological saline at 37° and Neubauer chamber was used as described by Belsey *et al.*, (1980). The epididymal plasma was used for analysis of total sperm count, motility and morphology (Abnormal sperm). 0.5ml of the epididymal fluid was added to 1ml of the semen diluting fluid (Sodium bicarbonate 5g, formalin 1ml, distilled water 99.0ml) for sperm count analysis and subsequently mixed. One drop of diluted epididymal fluid was added to the haemocytometer for 10min for the observation of sperm motility. In the same vein the number of spermatozoa in the appropriate squares of the haemocytometer was counted under microscope. The number of spermatozoa per ml of epididymal plasma was calculated according to the method described by Belsey *et al.*, (1980) as (No. of spermatozoa X dilution factor X depth factor/No. of area counted).

The relative proportions of abnormal sperm cells were analyzed by the technique described by Bauer *et al.*, (1974). Morphologically sperm cells were determined by taken an equal volume of cauda epididymal plasma and 5% NaHCO₃ and placed in a centrifuge tube, mixed well and centrifuge for 5min at 4000rpm. Supernatant was discarded and 5ml of normal saline was added to the precipitate, mixed well and centrifuge again until a clear precipitate was obtained. Few drops of normal saline were added to the final precipitate mixed thoroughly and smear was prepared on a clear slide, it was dried at room temperature. Fixed by heating over a flame for 2 sec then the smear was flushed with 95% alcohol, drained and dried. The smear was stained in Ziehl Neelson's Carbol Fuchsin diluted with equal volume of 95% alcohol for 3min. the smear was rinsed in water and dried in the air. The abnormal sperm cells were characterized based on the presence of double tail, detached head or tail and or irregular head.

For sperm viability, about 0.2ml of sperm sample was incubated with 1% trypan blue stain for 10min at 37°. A drop of the suspension was placed in a Neubauer haemocytometre chamber under a cover slip and allowed to settle for 1min and observed under microscope. The number of stained (blue) and unstained (colourless) spermatozoa which were not stained with trypan blue were considered as viable. This was based on the principle that damage plasma membrane, such as those found in dead cells allow entry of membrane-impermeant stains as described by Srinivasulu and Changamma, (2017).

The pH of the semen reflects the balance between pH values of the different accessory gland secretions mainly the alkaline seminal vesicular secretion and the acidic prostatic secretion. pH paper in the range of 5.0 to 10.0 was used in the determination of pH. The semen sample was thoroughly mixed, and a drop of the semen was spread evenly onto the pH paper for 30 sec. the result was later compared with the calibration strip on the pH meter as described by Ahmed *et al.*, (2013). Similarly blood samples were collected from the rats using a 2ml syringe through their veins between 8 am and 10 am. 2 mls of each blood sample was collected into clean EDTA free containers for biochemical analysis. The samples were earlier allowed to coagulate to produce sera for the blood analyses of glucose, cholesterol, total protein, albumin, urea and creatinine. Cholesterol was analyzed using commercial kit (Labkit chemex S. A. Spain). However, total protein and albumin were measured using (Quinica clinica kit Spain) as described by Wootton (1964). Urea was measured by blood urea nitrogen detection kit (Stressmarq Canada) and creatinine with serum creatinine detection kit (DetectX).

Statistical Analyses

All data generated from the study were subjected to analysis of variance (ANOVA) described by Steel and Torrie (1980) using statistical package of SPSS version 24.0.0.0 of 2010. Means were separated using

Duncan's Multiple Range Test where significant difference exist Duncan, (1980).

Result and Discussions

Proximate Composition of Raw and Toasted *Jatropha curcas* Seeds

Table 2 shows the proximate composition of both raw and toasted *Jatropha curcas*. It was observed that toasted *Jatropha curcas* has higher amount of crude protein (33.45%) while the raw/ unprocessed *Jatropha curcas* has lower value of crude protein (29.02%). The increase in protein contents of toasted sample also might be attributed to an increase in the free nitrogen content after toasting (Olawepo *et al.*, 2014). For the crude fibre, toasted sample has higher percentage of crude fibre (3.12%) while the raw had lower value recorded (2.20%). The raw /unprocessed *Jatropha curcas* had moisture content of (10.20%) slightly higher than (10.17) for the toasted. which is obviously due to drying effect (Ojo, *et al.*, 2015). The dry matter (DM) content value of raw and toasted *Jatropha* seed were 89.80 and 89.83%, similar with the range values of 89.20% reported by Oladunjoye *et al.*, (2014) for *Jatropha* seed cake, but lower than the range of 94.24 - 97.71 reported by Ojediran *et al.* (2014) for raw and toasted *Jatropha* seed meal. These differences could be due to defatting process as reported by (Ojediran *et al.*, 2014). The nitrogen free extracts (NFE) for raw *jatropha* seeds were 42.03% and 40.33% for toasted. This values obtained were higher than the 33.81 and 29.42 reported by (Antyve *et al.*, 2017) for raw and toasted *Jatropha* seeds meal. The raw *Jatropha* has the highest metabolizable energy (ME) of 3485.94 kcal/kg compared to the toasted. This could be as a result of high ether extract in the raw seeds.

Proximate Composition of Experimental Diet

The proximate composition of the experimental diets as presented in Table 3 showed that the crude protein (CP) values ranged from 20.14 to 20.25%. These values fall within the recommended protein

requirement values of 20-23% (Oluyemi and Roberts, 2000) and 22-25% (Olomu, 2011). The crude fibre (CF) content of the experimental diets ranged from 6.28 to 6.82%. This was higher than the 2.70% recommended for rats (Olomu, 2011). The metabolizable energy (ME) ranged from 3025.25 to 3060.68 kcal/kg this agrees with the 3193.00 kcal/kg reported by (Agboola and Adenuga, 2015).

Some Anti-nutritional Compounds in *Jatropha curcas* Seeds

The major problem of using *Jatropha* seed is its high content of some anti-nutritional compounds/inhibitor like tannins, phytate, saponins and phenols. Table 4 shows some of the anti-nutritional compounds found in *Jatropha curcas*. The raw *Jatropha* proved to have higher anti-nutritional factors than the processed *jatropha* for all anti-nutritional factors tested. For instance, raw *Jatropha* recorded higher values of tanins (+53.45%), phytates (+34.93%), saponins (+45.89), alkaloids (+12.98%) and phenols (+108.45%) compared to the toasted *Jatropha* which recorded lower values (+14.23), (+22.58%), (+13.77%), (+6.53%) and (+35.87%) respectively. toasting has drastically reduced the effect of anti-nutritional factors in toasting *Jatropha* seeds showing significant reductions in all the anti-nutritional content of *Jatropha curcas* seeds, and this indicates high protein which is an important nutrient in reproduction generally. Foruie *et al.*, (2004) reported that protein plays a major role in many aspects of male properties including the attainment of body weight, sexual maturity and onset of puberty. This justifies the increase in sperm count with toasted *Jatropha* having high protein content in the present study. It also implies that *Jatropha* seed has a high potential to complement or partially substitute groundnut cake as a protein source in diets of animals (Makker *et al.*, 2008).

Effects of Experimental Diets on the Semen Quality of Albino Rats

Sperm tests provide a direct measure of the quality of sperm produced in animals under

observation (Etta *et al.*, 2012). According to Sarathchandiran *et al.* (2007), lack of motility, decrease sperm count and incident of sperm abnormalities may be related to spermatotoxic effect of any herb extract via epididymis. The information in Table 5 showed that toasted *Jatropha* seed meal has significantly increased ($P<0.01$) in semen motility (active), morphology ($P<0.001$), pH ($P<0.01$), sperm count ($P<0.001$), and sperm density ($P<0.001$) 53.33%, 90.11%, 6.28, 3.10 and 544.4% respectively when compared with the values obtained from their respective control as 20.0%, 50.0%, 4.67, 1.09% and 176.67% which is in agreement with the reports of Oyawoye and Ogunkunle (1998). This further suggests that, toasted *Jatropha* seed meal had positive effect on all the parameters taken except for semen viability and non – motility. which are non-significant ($P>0.05$) and not in agreement with the report of Oyeyemi *et al.* (2011), who observed non-significant difference ($P>0.05$) in some of the semen characteristics of West Africa Dwarf (WAD) rams supplemented with *Euphorbia hirta*. They used an extract containing tannins, alkaloids and phenols which are also found in *Jatropha curcas* seed used in the present study. Contrary to this, other studies suggest that, ethanolic extract of *Jatropha gossypifolia* caused a significant decrease in testosterone levels and sperm motility, thus suggesting that the extract inhibited spermatogenesis mechanism by intervening with the process of hormone synthesis in the Leydig cells with a resultant alteration in the micro environment of the seminiferous tubules as reported by Oyedaji *et al.* (2013). Srinivasulu and Changamma (2017) however, reported an insignificant decrease in sperm viability as well as a significant increase in the percentage of morphologically abnormal sperm cells induced after treatment of rats with the *Ocimum sectum* leaf extracts. These reports are not in conformity with the present study, as the result showed significant ($P<0.001$) increase in sperm count and sperm motility. The result also agrees with the report of Antyev *et al.* (2017) that reported

high crude protein value in toasted *Jatropha* seed meal, which aid significantly in spermatogenesis.

Effect of the Experimental Diets on Serum Biochemistry of Albino Rats

Table 6 shows the results of serum biochemical indices values of rats fed with toasted *Jatropha* seed meal. The values have indicated that, serum urea was significantly ($P<0.05$) affected with the highest value in T3 (3.68%) and T2 (3.05%). This can be attributed to the sudden introduction of *Jatropha* to the system of the albino rats. Increase in serum urea agrees with the findings of Antyev *et al.*, (2017) and Esonu *et al.*, (2001) who reported that serum urea and total protein content depends on both the quality and quantity of the protein supplied in the diet. This is not out of the range of (12.3 to 24.6 mg/dl) as reported by Mary and Charles in Chemical Laboratory Parameters reference book, (2008). Treatments T2 and T4 appear to function simultaneously in relation to blood cholesterol which was reduced. Other treatments indicated high cholesterol levels which may be attributed to the oil that was not removed in the *Jatropha* seeds.

Conclusion

It could therefore be concluded that, semen quality may be improve leading to improvement in fertility when toasted *Jatropha* seed meal is incorporated in to the diet of albino rats without coursing harmful effects on serum biochemical indices. *Jatropha* seed meal may also be used as an alternative to GNC (protein source), as it is readily available and cheaper than GNC.

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Table 1: Feed Composition of the Study Diets with Replacement Levels of TJSM for GNC

Ingredients	(0%)	(2.5%)	(5%)	(7.5%)	(10%)
Maize	49	49	49	49	49
GNC	20	19.5	19	18.5	18
Jatropha	0.0	2.5	5.0	7.5	10
Wheat offal	26	24	21	20	18
Fish meal	2.0	2.0	2.0	2.0	2.0
Bone meal	2.2	2.2	2.2	2.2	2.2
Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25
Calculated analysis					
Crude Protein	18.16	18.10	18.04	17.98	17.92
Ether Extract	4.49	4.51	4.52	4.54	4.56
Crude Fibre	5.12	5.11	5.11	5.11	5.10
Calcium	1.05	1.04	1.04	1.04	1.04
Phosphorus	1.96	1.96	1.96	1.96	1.96
Methionine	0.37	0.37	0.37	0.37	0.36
Lysine	0.58	0.57	0.56	0.78	0.55

Mineral premix / kg the following: Vit A 1500 IU; Vit D3 3000 IU; Vit E; 30 IU; Vit K, 2.5mg; Thiamine B1 3mg; Riboflavin B2 6mg; pyridoxine B6 4mg; Niacin 40mg; Vit B12 0.02mg; pantothenic acid 10mg, Folic acid 1mg; Biotin 0.08g; Chloride 0.125g; Mn 0.096g; Antioxidant 0.125g; Zn 0.06g; Fe 0.024g; Cu 0.006g; Se 0.24g; Co0.240g. **Key:** TJSM= Toasted Jatropha seed meal GNC= Groundnut cake.

Table 2: Proximate Composition of Raw and Toasted Jatropha seeds

Nutrients	Raw	Toasted
Crude Protein	29.02	33.45
ME (kcal/kg)	3485.1	3445.3
Dry matter	89.80	89.83
Ash	5.20	3.35
Ether Extract	11.35	9.58
Crude Fibre	2.20	3.12
Nitrogen Free Extract	42.03	40.33
Moisture	10.20	10.17

Table 3: Proximate Composition of the Experimental Diets

Composition	T1 (0%)	T2 (2.5%)	T3 (5%)	T4 (7.5%)	T5 (10%)
Crude Protein	20.14	20.20	20.20	20.21	20.25
ME (Kcal/kg)	3060.6	3038.8	3060.0	3040.7	3025.2
Moisture	10.50	10.60	10.40	10.35	10.45
Dry matter	89.50	89.40	89.60	89.45	89.55
Crude Fibre	6.28	6.70	6.31	6.82	6.70
Ash	6.57	6.86	6.93	7.13	7.60
Ether Extract	6.80	6.95	7.01	7.10	7.11
NFE	49.71	48.69	49.15	48.39	47.89

Table 4: Phytochemistry of *Jatropha curcas* Seed

Phytochemical Compounds	Raw	Toasted
Tannins	+55.45	+14.23
Saponins	+45.89	+13.77
Phenols	+108.45	+35.89
Alkaloids	+12.98	+6.53
Glycosides	+15.88	+3.55
Phytates	+34.92	+22.58

Key: + = Positive

Table 5: Semen Quality Characteristics of Albino Rats Fed Toasted *Jatropha* Seed Meal

Parameter	T1 (0%)	T2 (2.5%)	T3 (5%)	T4 (7.5%)	T5 (10%)	SEM
Motility (Active)	20.00 ^c	30.00 ^{bc}	23.33 ^c	43.33 ^{ab}	53.33 ^a	5.16 ^{**}
Motility (Slow)	30.00	33.33	33.33	33.33	23.33	7.67 ^{NS}
Non-motile	50.00	36.66	46.66	23.33	20.00	11.00 ^{NS}
Viability	29.00	50.00	41.11	49.24	40.55	8.37 ^{NS}
Morphology	50.00 ^c	79.44 ^b	87.78 ^a	90.93 ^a	90.11 ^a	1.35 ^{***}
pH	4.67 ^b	6.28 ^a	5.55 ^{ab}	5.89 ^{ab}	5.89 ^{ab}	0.44 ^{**}
Sperm count	1.09 ^c	1.72 ^b	3.10 ^a	1.63 ^{bc}	2.00 ^b	0.17 ^{***}
Sperm density	176.67 ^c	462.22 ^{ab}	519.78 ^{ab}	413.00 ^b	544.4 ^a	34.78 ^{***}

Means on the same row with different superscripts are significantly different* = (P<0.05) ** = (P<0.01) *** = (P<0.001) SEM – Standard Error of Mean, NS=Not Significant

Table 6: Biochemical indices of Albino Rats Fed Toasted Jatropha Seed Meal

Parameter	T1 (0%)	T2 (2.5%)	T3 (5%)	T4 (7.5%)	T5 (10%)	SEM
Biochemical Indices						
Glucose	14.25	15.23	18.13	17.03	13.33	1.70 ^{NS}
Cholesterol	316.25 ^b	314.74 ^b	324.25 ^a	314.00 ^b	317.00 ^b	1.93*
Protein	5.35	4.73	5.23	5.00	4.83	0.28 ^{NS}
Albumin	3.58	3.30	3.38	3.53	3.38	0.11 ^{NS}
Urea	2.83 ^{ab}	3.05 ^{ab}	3.68 ^a	2.80 ^{ab}	2.50 ^b	0.39*
Creatinine	59.00 ^{bc}	109.00 ^a	65.50 ^{bc}	93.25 ^{ab}	41.75 ^c	13.72*

Means on the same row with different superscripts are significantly different*=(P<0.05) SEM – Standard Error of Mean NS=Not Significant.