

Full Length Research Paper

Nutritive composition of omega-3 fatty acids-rich *Ricinodendron heudelotii* and its potential for nutrition

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Njangsa (*Ricinodendron heudelotii*), a tropical tree that grows in the forest of West and Central Africa, produces fruits that are manually shelled to collect oil seeds and dried. A compositional analysis of njangsa revealed a unique nutrient presence of long chain omega-3 fatty acids not usually associated with plant materials. The seed had 31.4% crude protein and 44.7% lipid. Of this lipid, about 73% was composed of polyunsaturated fatty acids (PUFA), almost entirely of eicosapentaenoic acid, with about 18% oleic acid. Preliminary studies were conducted to determine if njangsa seed meal would alter the lipid and other metabolite levels in the pig and/or improve pork quality traits. Twelve crossbred gilts and barrows were fed corn-soybean diets containing 14% crude protein. Treatment group was supplemented with 2% njangsa oil seed meal. Growth and carcass traits showed similar carcass characteristics ($P > 0.05$). Backfat measurement was reduced ($P < 0.05$), while kidney weight was elevated ($P < 0.01$) in treated animals. Pork sensory evaluations were not different between the experimental groups. Oil rich supply of long chain PUFA from sources other than seafood may provide a more sustainable source.

Key words: *Ricinodendron heudelotii*, fatty acids, eicosapentaenoic, docosahexaenoic, swine, carcass composition.

INTRODUCTION

Ricinodendron heudelotii, commonly known as njangsa, is a fast growing late secondary tropical tree found in Guinean-Congolese humid forest of west and central Africa. *R. heudelotii* reaches maturity between 4 to 5 years before producing fruits. The fruits are usually manually shelled to collect the oil seeds. The njangsa seed is extracted from the hard shell through a crude process which involves boiling the kernel for hours at high temperature above 100°C.

This process subjects the kernel to a high temperature which often results to oxidation and rancidity of the oil. The seed is valued for its distinctive flavor. The seeds are dried, ground and used for soup and as an ingredient for seasoning baked meats and fish, and flavoring and thickening agent in food (Plenderleith, 1997). The oil seed is an economical and valuable agricultural plant, especially in Cameroon. *R. heudelotii* is a source of many Nutrients and biologically active compounds that include

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Table 1. Swine finisher diet composition (CP 14%) fed to growing pigs.

Ingredient (%)	Experimental treatment	
	Control	Njangsa
Corn	83.79	82.42
Soybean	12.2	12.46
Njangsa	-	2.0
Tallow	1.89	1
VitaminPremix ^a	0.25	0.25
Salt	0.25	0.25
Dical PO ₄	0.71	0.71
Limestone	0.9	0.9
Antibiotics	0.01	0.01
Total	100	100
ME (Kcal/kg)	3275	3318

^aProvided (per kilogram of Premix) 2,204,585.5 IU Vit A; 440,917 Vit D₃; 4,409.2 IU Vit E; 6.7 mg Vit B₁₂; 222.2 mg Menadione; 38,553 mg Choline; 5,555 mg Niacin; 3,777.8 mg D-Pantothenic acid 1,222.2 mg Riboflavin; and 411 mg Thiamin provided (% of Premix).

omega-3 fatty acids, essential amino acids, minerals and antioxidant vitamins (Besong et al., 2010).

Njangsa oil seed is widely used in West Africa as food and has medicinal as well as industrial applications (Mori et al., 1999; Orwa et al., 2009; Ekam, 2003). The leaves are used as important source of high-quality fodder for sheep and goats in the dry season. It was discovered that the green foliage had average crude protein content of 16% and without any known toxicity (Anigbogu, 1996; Latham, 1999). However, presence of unnamed traces of an alkaloid and resin was reported to be contained in the seed (The World Agroforestry Centre (ICRAF), 1999). Manga et al. (1990) reported that total fat content of *R. heudelotii* kernels from various locations in Cameroons ranged from 50 to 65.2%. This compared favorably with the oil content of commercial vegetable oils reported by Zambiasi et al. (2007). Fatty acid composition showed a high level of polyunsaturated fatty acids (PUFA) (C_{18:3}) and essential amino acids (Tehiegang et al., 1997). Crude protein level ranged from 49.9 to 65.2% (Manga et al., 2000).

The presence of longer chain omega-3 fatty acids in *R. heudelotii* oil seeds would create a greater need for exploitation of this plant for human and animal nutrition. There is an increasing trend towards producing leaner pork from dietary or genetic improvement methods by altering the fatty acid profile resulting in healthier market products. This trend has resulted in producing pork with reduced subcutaneous fat and ideal amount of intramuscular lipids. Previous studies have shown that these desired qualities may be accomplished by low protein diets (Doran et al., 2006); patents for enriching lean meat with omega-3 fatty acids exist, which creates a future possibility for meat providing appreciable quantities of these fatty acids. Omega-3 fatty enriched lean meat

has the potential to expand the feed industry's market for omega-3 enriched rations (Nettleton, 1995). The objectives of this study were to determine the nutritive composition of njangsa oil seed and its effect on lipid and other metabolites in pigs fed supplements of the oil seed meal. Meat quality and carcass traits including shelf life of meat from njangsa fed animals were determined.

MATERIALS AND METHODS

Sixty kilograms of dried njangsa seeds were purchased from limited resource farmers in the Western Region of Cameroon, West Africa. Extracted seeds were dried and kept at room temperature and later placed in Ziploc bags prior to shipment to the United States. Upon receipt in our lab, the seeds were stored at 4°C until used for feeding trial. The seeds were blended and mixed to yield oil and seed meal. The dried seeds were "full fat" seeds, lyophilized and ground prior to mixing into rations. Two feed samples were analyzed for nutrient composition (Covance Lab Inc., Madison, WI)

Diet and experimental design

Twelve crossbred gilts (163.3 kg) were obtained and housed at Alcorn State University Swine Research and Development farm. The animal experimental protocols were reviewed and approved by Alcorn State University Animal Care Committee. These pigs were randomized according to body weight and placed into two groups and housed at separated pens. The two groups were allotted into either the control or njangsa fed groups. Pigs were fed 14% crude protein from corn-soybean meal diet supplemented with either 2% njangsa meal (treatment group n = 6 per group) or control diet (no njangsa) group (n = 6 per replicate) and formulated to meet the nutrient requirement for finishing pigs (NRC, 1988). Diets were fed *ad libitum* and feed intake was calculated on weekly basis. All diets were isocaloric and isonitrogenous (Table 1). A two-week acclimation period was followed before the six weeks experimental treatment. Weekly body weight and blood samples were collected for analysis of total cholesterol and triglycerides. All pigs were slaughtered at the end of experiment at USDA Meat Inspection Facility. Liver, kidneys and gastrocnemius muscles were dissected and weighed.

Analytical methods of njangsa seeds, blood and animal tissues

Five grams of ground samples were taken for crude protein and total ash extractions (Association of Analytical Communities (AOAC), 1995). Total lipids were determined by the (Sukhija and Palmquist, 1988) one-step methylation method using hexane as a solvent. Fatty acid and amino acid compositions were determined by gas chromatographic and high performance liquid chromatography (HPLC) techniques, respectively (Midwest Laboratories Omaha, NE). Vitamins A and α -tocopherols in pork muscles (gastrocnemius) and feed were analyzed by Convince Laboratories (Madison, WI).

Analysis of blood and animal tissue

Serum cholesterol and triglycerides were analyzed using Wako Diagnostics kits (Wako Diagnostics, Richmond, VA). Total lipids and cholesterol in skeletal muscles, liver tissues and triglycerides were extracted and quantified (AOAC, 1998).

Table 2. Chemical Composition and antioxidant vitamins in *R. heudelotii* seed meal fed to growing pigs.

Item	Meal ⁿ¹
Crude protein, %	31.4
Total lipid, %	44.7
Saturated fatty acids, %	13.5
Monounsaturated fatty acids, %	12.8
Polyunsaturated fatty acids, %	73.7
Trans fatty acids, %	n.d
Vitamin A, IU/100 g	192
Vitamin E, IU/100 g	2.41

n=2. ¹Analyzed by Convance Lab Inc. Madison, WI.

Statistical analysis

Data analyzed statistically using Statistix-7 for Windows, Analytical Software 2002. Body weight, daily gain, feed intake, and efficiency (gain/feed) were analyzed as six experimental units per treatment using Statistix-7 for Windows, Analytical Software 2002. A 2 × 6 factorial analysis of variance was used to determine the effect of diet treatment and bleeding times on blood metabolites and lipid levels. Treatment means were separated by the least significant difference (LSD) technique.

RESULTS AND DISCUSSION

The chemical composition of njangsa seed meal showed a remarkable nutritive quality previously not reported from plant sources (Table 2). The crude protein content compares well with other well-known oil seeds such as peanut and soybean meals. Plant protein sources were reported to enhance the hypocholesterolemic effects of dietary polyunsaturated fatty acids (Forsythie et al., 1980). The unique lipid composition of njangsa is remarkably different from others reported for plants. Recent discovery in our lab showed that njangsa oil seeds (*R. heudelotii*) (Figure 2) have high levels of polyunsaturated FA and are rich with substantial amounts omega-3 fatty acids, especially eicosapentaenoic acid (EPA), not found in other oil seed meals (Table 3). EPA and docosahexaenoic acid (DHA) are usually associated with marine fish oils and have not been reported in plant species at high concentration observed in this study (Table 3). Our results showed that njangsa oil is a potential alternative to fish oil and appears to be a promising land-based source for omega-3 fatty acids, in contrast to the findings of Tchankou Leudeu et al. (2009). The differences may be due to the differences in sensitivity of the analytical methods used. Njangsa oil appears to be superior to all commercial vegetable oils that are used for cooking (Table 3). Previous chemical composition analysis indicated crude protein range of 49.9 to 65.2% and 49.3 to 63.5% fat in 47 collections of njangsa seeds from different regions in Cameroon (Manga et al., 1999). These results are in agreement with those reported in the present studies. However, the presence

of high amount of PUFA was not expected. The identification of EPA and DHA for the first time in significant amount is bound to have a beneficial and positive effect on human health and animal nutrition. EPA supports cardiovascular, immune and other systems and is important to human health (Pakala et al., 1999). Linolenic acid in most instances must be converted to long chain unsaturated omega-3 fatty acids such as EPA and DHA before it can be metabolically useful to humans and animals (Nettleton, 1965). Omega-3 fatty acids are taken up by many cells and tissues of animals, including plasma, liver, lung, kidney and spleen, aorta, vascular endothelium, heart, and tumors. Fatty acids, particularly DHA are concentrated in the membrane phospholipids of the retina and brain, while EPA is preferentially distributed in liver, kidney, platelets and blood cells (Nettleton, 1995). Pigs in the njangsa diet had a significant ($P < 0.01$) increase in kidney size with no prior adverse health problems. However, it was difficult to determine the cause of the differences in kidney size from this study. Our results suggest that incorporation of njangsa seeds in the diet would provide a good source of omega-3 fatty acids which are lacking in the Western diet. The American Heart Association (AHA) and National Academy of Sciences and Institute of Medicine have recently made dietary recommendations focusing on substituting EPA and DHA for saturated fatty acids.

Table 4 presents data on the amino acid content of njangsa oil seed. While low in lysine and tryptophan, the percentage of essential amino acids to total amino acids was 40.59% which was higher than normal values for a well-balanced protein feed (Tehiegang et al., 1998). Njangsa oil seed had a good balance and concentration of other essential amino acids. In view of the spread of this tropical tree in west and central Africa, the importance of developing the production of the seeds as a sustainable source of omega-3 fatty acids for animals and human consumption cannot be overemphasized. Strategies for the domestication of *R. heudelotii* have been suggested (Ngo Mpeck et al., 2003).

Carcass composition data in pigs are presented in Table 5. There was a significant reduction ($P < 0.05$) in back fat of pigs supplemented with 2% *R. heudelotii* meal but no differences were observed in other carcass measurements. Furthermore, it is noteworthy that no visible lesions were observed among the organs and tissues of the carcass. Liver, muscle and serum studied did not show improved storage of polyunsaturated fatty acids (EPA and DHA) or a reduction in cholesterol and triglycerides (data not presented). Stewart et al. (2001) observed that a diet containing modified pork with high polyunsaturated fatty acids (PUFA) significantly lowered total plasma and LDL-cholesterol in women, thus suggesting a new approach for lowering the consumption of saturated fat and improve the quality of pork products. Other studies showed that freeze-dried purslane leaves containing high levels of omega-3 fatty acids reduced blood total cholesterol and LDL-cholesterol in humans

Table 3. Fatty acids composition in commercial vegetable oil and njangsa oil seed (*Ricinodendron heudelotii*).

Fatty acid %	linseed ¹	Canola ¹	Soybean ¹	Corn ¹	Sunflower ¹	Peanut ¹	Olive ¹	NOS ²
Palmitic (C16:0)	4.81	3.75	9.77	10.41	5.73	9.4	10.84	18.9
Stearic (C18:0)	3.03	1.87	4.16	2.03	4.78	2.65	3.59	15.2
Palmitoleic (C16:1)	nd	0.21	0.06	nd	0.03	0.06	0.92	0.16
Oleic (C18:1, cis)	21.42	62.41	22.4	24.9	15.81	48.71	75.55	6.89
Linoleic (C18:2, cis)	15.18	20.12	54.47	59.83	70.93	31.06	7.01	24.9
Alpha linolenic (C18:3)	54.24	8.37	7.38	1.03	0.37	0.23	0.66	0.8
Eicosapentaenoic (C20:5)	nd	nd	nd	nd	nd	nd	nd	48.6
Docosahexaenoic (C22:6)	nd	nd	nd	nd	nd	nd	nd	0.7

¹Data provided by Zambiasi et al. (2007). ²Njangsa oilseed (*Ricinodendron heudelotii*).²Njangsa oilseed (n = 2): Analyzed by Convince Lab, Madison, WI.

Table 4. Essential amino acid composition of njangsa mealⁿ and soybean mealⁿ

Essential amino acids	¹ Njangsa meal (%)	² Soybean meal (%)
Arginine	3.5	3.17
Histidine	0.61	1.26
Isoleucine	1.1	1.96
Leucine	1.6	3.43
Lysine	0.7	2.76
Methionine	0.58	0.6
Phenylalanine	1.26	2.26
Tryptophan	0.1	0.59
Threonine	1.05	1.76
Non-essential amino-acids		
Alanine	1.3	-
Aspartic Acid	2.5	-
Cystine	0.97	-
Glutamic Acid	4.21	-
Glycine	1.4	-
Proline	1.2	-
Tyrosine	0.75	-
Serine	1.5	-

n=2. ¹Analyzed by Midwest Lab, Omaha, NE. ²Cromwell et al. (1999). Journal of Animal Science.

and pigs (Besong et al., 2011). It is not clear why *R. heudelotii* oil seed meal fed to pigs did not influence tissue lipids in the present study. The level of PUFA supplied by *R. heudelotii* (2% of total diet) might have been completely metabolized by the impacted organs and tissues (kidney and adipose tissue), and not much was left to influence other tissues. Reports have shown that substitution of 15% lipids in diets with PUFA concentrate prevented fat accumulation with preferential reductions in abdominal fat depot in mice (Ruzickova et al., 2004; Flachs et al., 2005). These studies led to the suggestion that reduction in both hyperplasia of adipose tissue cells and hypertrophy of adiposities contribute to

reduced accumulation of body fat as a result of PUFA intake (Kopecky et al., 2009), agreeing with the result of the present study.

The sensory evaluation of pork from treated and control animals did not show any significant ($P > 0.05$) differences in flavor, juiciness, tenderness and texture (Figure 1). Addition of PUFA-rich diet used in the present study did not adversely affect the quality of pork. It appears that the higher level of antioxidant vitamins present in njangsa seed meal (Table 6) may have contributed to improvement in pork's nutritional quality. High levels of vitamin A in meat after long term storage may have contributed to enhanced shelf life in meats from treated animals (Table 6).

Table 5. Growth and carcass traits of finishing hogs supplemented with novel feed additive *Ricinodendron heudelotii* (njangsa)¹

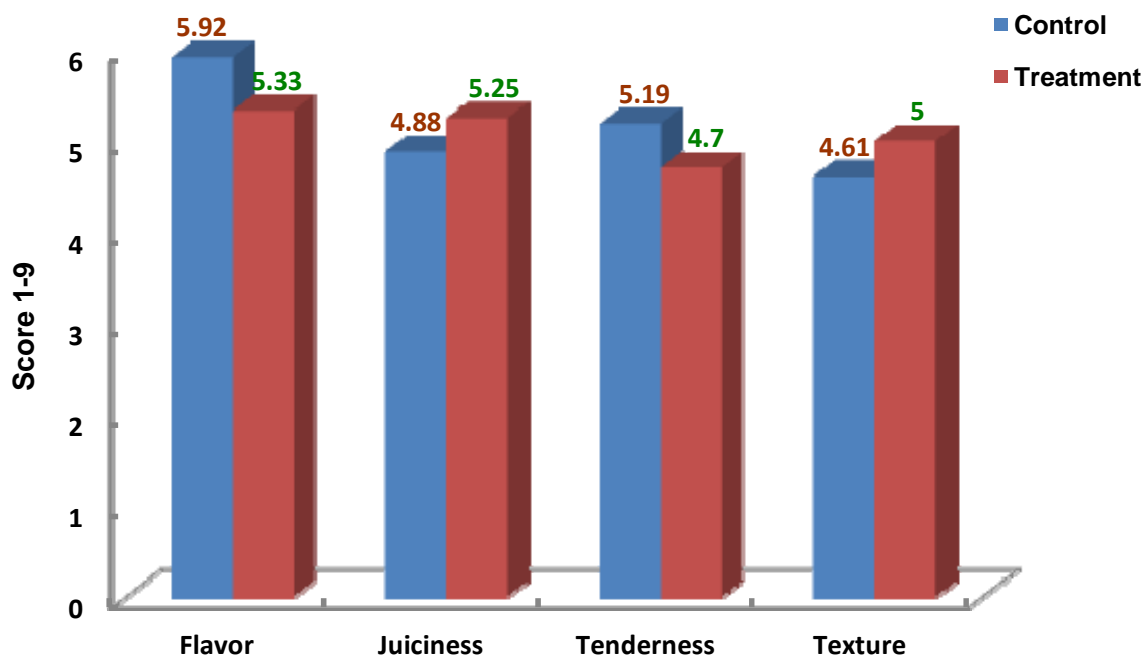
Trait	Treatment	Control	Treatment vs. control (P-values)*
Start weight (kg)	67.38±7.9	68.53±6.0	0.75
End weight (kg)	110.75±18.2	111.36±11.0	0.93
Average daily gain (kg)	1.0±0.3	0.99±0.1	0.91
Slaughter weight (kg)	104.21±17.9	102.22±9.9	0.79
Dressing (%)	75.51±9.2	81.08±1.1	0.19
Cold carcass weight (kg)	79.72±14.7	80.20±7.4	0.97
Moisture loss (%)	3.13±0.3	3.23±0.3	0.66
Fat depth (cm)	1.68±0.1	2.44±0.05	0.05
Loin eye area (cm ²)	88.65±0.45	88.49±0.2	0.97
Liver (kg)	64.56±0.3	64.07±0.2	0.92
Kidney (kg)	0.29±0.03	0.24±0.02	0.008
Heart (kg)	0.41±0.05	0.38±0.02	0.27

¹Means ± SD. for 6 animals. *Significant if P<0.05

Table 6. Effect of long-term (3 years) storage of gastrocnemius muscle of pigs fed *R. heudelotii* on Vitamins A and E¹ level.

Item (mg/100 g)	Treatment	
	Control	Njansa
Vitamin A	3.2±1.4 ^a	6.0±1.5 ^b
Vitamin E	326.4±107.9 ^a	360.0±187.8 ^a

^{ab}Means within each row with different superscript differ (P<0.05).

**Figure 1.** Pork sensory (taste panel): Evaluation of latissimus dorsi from pigs fed *R. heudelotii* meal.

^aMeans. Control and treatment values did not differ (P > 0.05).



Figure 2. Oil seeds extracted from njangsa kernel.

A two-fold ($P < 0.05$) increase in vitamin A level in meat indicates efficient absorption and storage from feed sources by pigs.

Conclusion

The quantity and quality of polyunsaturated fatty acids (PUFA) in *R. heudelotii* seeds indicate an unusual presence of EPA in plant species, suggesting a potential land-based source of omega-3 fatty acids. The presence of high amount vitamins A and E in njangsa seed suggests a potential source of dietary antioxidant nutrients and may also provide a protective effect on PUFA in the seed. The study showed that incorporating *R. heudelotii* oil seed meal into swine diet significantly reduced back-fat thickness by 31.1% and an elevated kidney weight by 20.8%. High levels of antioxidant vitamins reflected in meat after long term storage indicate a possible improvement in shelf-life of meat. More research is needed before consumers will be able to benefit from food/meat products enriched with antioxidant vitamins and PUFA from *R. heudelotii* for animal and human nutrition. Results from the swine feeding trial

showed a shift in fat deposition toward a reduction in back-fat and elevated vitamin A concentration in meats. A great potential exists for use of *R. heudelotii* oil seeds for meat quality improvements and enhancement of human health and nutrition.

Conflict of interest

Authors declare that there are no conflicts of interest.

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