Original Paper

Nutritional and phytogenic properties of pawpaw (*Carica papaya*) leaf meal on blood characteristics of growing rabbits

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The study aimed to examine the effect of pawpaw (*Carica papaya*) leaf meal diets on blood characteristics of rabbits. A total of 48, male rabbits were randomly divided into four experimental groups of twelve animals each, with four rabbits constituting a replicate. Each group was assigned to one of the experimental diets containing pawpaw leaf meal (PLM) at 0% (control), 15%, 30% and 45% for 56 days in a completely randomized design (CRD). Blood samples for analysis were obtained from each replicate and data obtained were analyzed statistically. Results on chemical composition of the PLM revealed 87.67% DM, 17.30% CP, 12.86% CF, 8.88% ash, 0.81% EE 47.82% NFE and 2348.05 Kcal/kg ME. PLM at 15% inclusion increased (P <0.05) the packed cell volume (PCV) and haemoglobin (Hb) when compared with the control. PLM at 30 and 45% resulted to improved mean cell volume (MCV) concentration. The concentrations of white blood cells were increased (P <0.05) at the treatment (15%, 30% and 45%) groups. Red blood cell, mean cell volume, mean cell haemoglobin concentration, creatinine, AST, ALP, sodium, potassium and chloride remained similar (P >0.05) across the treatments. Total protein was however best (P <0.05) at 45% supplementation. The results indicated that PLM enhanced haemopoiesis and health status of the experimental rabbits and therefore should be incorporated into rabbit feeding to enhance blood formation and health status of the animals.

Keywords: rabbits, pawpaw leaf, phytogenic compound, haematology and serum biochemistry

1 Introduction

Phytogenic compounds have recently received great attention in animal nutrition due to their growth-promoting and medicinal properties. Their uses in animal production have been on increase mainly by the belief that they are free from chemical additives and absence of toxic influence on the animals. However, incidences of toxicity have been reported by Edeh (2013) and Ogbuewu et al. (2014) among animals fed diets containing phytogenic compounds. Hence, the need to ascertain the nutritional and phytogenic properties of this plant materials before recommending their use in animal production. The clinical examination of blood becomes necessary since it provides reliable information about feed toxicity on animals fed such diets.

Pawpaw is known with diverse names, in Nigeria, it is known as "Okwuru bekee" by Igbos, "Ibepe" in Yoruba and "Gwada" by Hausas. Pawpaw is a herbaceous plant, with a single stem growing from 5 to 10 m tall, with spirally arranged leaves confined to the top of the trunk. The leaves are spirally arranged, clustered near the trunk apex, hollow, greenish or purplish-green, 50–75 cm in diameter, palmate, deeply 7-lobed, prominently veined and broadly toothed. The flowers appear on the axils of the leaves, maturing into large fruit. The fruit is ripe when it feels soft and its skin has turned yellowish-brown to orange shade. Pawpaw though a native of America is easily grown in Africa. Pawpaw Aravind et al. (2013) was reported to be a powerhouse of nutrients. The leaves contain carbohydrates, minerals and vitamins, lipids and proteins (Patil et al., 2014). The leaves are high in papain, volatile oil, terpenoids, folic acid, vitamins B_1, B_2, B_{12}, A, C and E, alkaloids (carpaine and pseudocarpaine), saponins, anthraquinones, cardiac glycosides, glucosinolate

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(benzylglucosinolate), choline, flavonoids, calcium, magnesium, sodium, potassium, manganese and iron. Pawpaw aids in digestion of protein at acid, alkaline or neutral medium due to the presence of papain, a natural enzyme, which also helps in the cleansing of gastro intestinal tract (Jiwuba, 2018). Earlier reports by Otsuki et al. (2010) and Nguyen et al. (2013) indicated that the leaves can prevent and kill cancer cells. It was also reported (Chávez-Quinta et al., 2011; Aravind et al., 2013; Nguyen et al., 2014; Patil et al., 2014) to have antimalarial, antiplasmodial, anthelmintic, antiparasitic, antibacterial, antiviral, antifungal, anti-inflammatory, digestive stimulant and antihypertensive activities. These thus indicated the dual properties of pawpaw leaves as a nutritional agent and medicinal agent; hence highlighting its nutritional and ethno-veterinary properties. Despite, these properties, pawpaw leaf meal has not been extensively considered as a reliable feedstuff for rabbits. The study was therefore, designed to evaluate the blood characteristics of growing rabbits fed varying levels of pawpaw leaf meal containing diets with a view of ascertaining nutritional and ethno-veterinary properties for improved rabbit performance.

2 Material and methods

2.1 Location of experiment

The experiment was carried out at the Rabbit Unit, Federal College of Agriculture, Ishiagu, Ebonyi state, Nigeria. The College is located at about three kilometers (3 km) away from Ishiagu main town. The College is situated at latitude 5.56 °N and longitude 7.31 °E, with an average rainfall of 1653 mm and a prevailing temperature condition of 28.5 °C and relative humidity of about 80% (Jiwuba et al., 2016a).

2.2 Sources and processing of experimental material

Fresh leaves of pawpaw were harvested within the College environment and air dried for some days to a moisture content of about 10%. The air dried leaves were processed and milled using 5 mm hammer mill. Other feed ingredients were procured from Farm associate, Enugu, Enugu State, Nigeria.

2.3 Experimental animals and management

Forty eight growing rabbits weighing averagely 477.01 g were randomly divided into four experimental groups of twelve animals each, with four rabbits constituting a replicate. Each rabbit was housed in a standard hutch measuring 120 by 150 cm and raised 120 cm above the ground level. The four treatment groups were assigned the four diets in a Completely Randomized Design (CRD). Each rabbit received an assigned diet for 56 days. Each animal was vaccinated against prevalent disease. They were also dewormed using kepromec (Ivermectin) at the rate of 0.1 ml per rabbit subcutaneously and given accaricide bath using Roys' Amitraz 20 at the rate of 1ml in 2 litre water prior to the experiment.

Experimental diet.Four (4) experimental diets were formulated and designated as T_1, T_2, T_3 and T_4 to contain pawpaw leaf meal (PLM) at 0%, 15%, 30% and 45%, respectively (Table 1). Treatment T_1 did not contain PLM thereby serving as the control diet.

2.4 Data collection

Blood samples (5 ml) were drawn from each animal on the last day of the study. The rabbits were bled through the ear marginal vein. The samples were separated into two lots and used for biochemical and haematological

Table 1 Percentage Composition of the experimental diets

Ingredient	T_1 (0%)	T_2 (15%)	T_3 (30%)	T_4 (45%)
Maize	43.00	42.00	40.00	38.00
Wheat offal	13.00	9.00	5.00	00.00
PKC	21.00	13.00	6.00	00.00
Fish meal	1.00	1.00	1.00	1.00
Soya bean meal	18.00	16.00	14.00	12.00
Pawpaw leaf meal	0.00	15.00	30.00	45.00
Bone meal	2.00	2.00	2.00	2.00
Lime stone	1.00	1.00	1.00	1.00
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25

studies. An initial 2.5 ml was collected from each sample in labelled sterile universal bottle containing 1.0 mg/ml ethyldiamine tetracetic acid and used for haematological analysis. Another 2.5 ml was collected over anti-coagulant free bottle. The blood was allowed to clot at room temperature and serum separated by centrifuging within three hours of collection. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyzer and Bayer DCA 2000+ HbA1c analyzer, respectively. Mean cells haemoglobin (MCH), MCV and mean cell haemoglobin concentrations (MCHC) were calculated.

2.5 Chemical analysis

All feeds and experimental material (PLM) were analyzed for proximate compositions using the method of AOAC (2000). The nitrogen free extract was derived by subtracting the sum of other proximate components, crude protein, crude fat, ash, crude fibre on dry weight basis from 100. Metabolizable energy calculated using the formula; ME = (3.5 × crude protein) + (8.5 × crude fat) + (3.5 × Nitrogen Free Extract) × 10.

2.6 Data analysis

The results were analyzed using the Statistical Package for Social Science Window 17.0. One-way analysis of variance (ANOVA) was employed to determine the means and standard error. Significant differences between the treatment means were separated using the Duncan Multiple New Range Test.

3 Results and disscussion

3.1 Proximate composition

The results of the proximate analysis of experimental diets and PLM are shown in Table 2. The dry matter (DM) range of 90.46–92.91% in this study compared well with the range of 90.95–93.29% reported by Jiwuba et al. (2016a) for weaner rabbits. The crude protein (CP) values in this study ranged between 16.70 and 17.66% which falls within the requirement for growing rabbits (16% CP) as recommended by NRC (1977) and Lebas (2013) and 16–18% as recommended by Fielding (1991). The crude fibre (CF) range of 15.40–16.45% reported in this study failed to follow a particular trend but however compared with the recommended values of 14–18% and 14–16% reported by Gidenne and Lebas (2002) and Mayer (1955) for growing rabbits. Adequate supply of dietary fibre reduces digestive problems, promotes intestinal motility and enhances growth in weaned rabbits. Hence, diets low in fibre promotes an increased incidence of intestinal problems, like enterotoxaemia and lower growth rates (Mayer, 1955; Gidenne and Jehl, 1999). The energy values reported in this study also failed to follow a particular trend. The reported range of 2,550.05–2,604.10 kcal/kg in this study is in agreement with the recommended values of 2,500.00kcal/kg, 2,400–2,800 kcal/kg and 2,500–2,800 Kcal/kg as recommended by NRC (1977), Pond et al. (1995) and Aduku and Olukosi (1990) respectively for growing rabbits. Rabbits however adjust their feed intake as a function of their dietary energy concentration. The ash values of 3.29–8.09% reported in this study followed a particular pattern increasing with increasing levels of PLM; an indication that the mineral requirements of the rabbits were met, since ash is a reflection of the mineral content of a diet. The highest value of ash observed in T_4 may be attributed to high levels of minerals which abound in pawpaw leaves. It is worthy to note that the values for the control diet compared favourably well with the treatment groups. The results of the proximate analysis of the PLM revealed 87.67% DM, 17.30% CP, 12.86% CF, 8.8 8% ash, 0.81% ether extract (EE), 47.82% NFE and 2,348.05 kcal/kg metabolisable energy. The DM content of 87.67% reported in this study is lower than 93.02% reported by Ganzon-Naret (2015), but however compared with 89.60% reported by Nath and Dutta (2016) for the same leaf meal. The crude protein value of 17.30% reported in this study is in agreement with 25.30, 32.60, 21.36 and 13.10% reported by Unigwe

Table 2 Proximate composition of the experimental diets and pawpaw leaf meal

Parameters (%)	Dietary levels (%)				
	T_1	T_2	T_3	T_4	PLM
Dry matter	92.12	90.82	90.46	92.91	87.67
Crude protein	17.44	16.70	16.84	16.70	17.30
Crude fibre	16.20	16.45	15.75	15.40	12.86
Ash	3.29	4.41	5.78	8.09	8.88
Ether extracts	2.30	3.11	2.75	2.65	0.81
Nitrogen free extract	52.89	50.15	49.34	50.07	47.82
Metabolizable energy (kcal/kg)	2,587.05	2,604.10	2,550.05	2,562.20	2,348.05

et al. (2014), Ogbuokiri et al. (2014), Ganzon-Naret (2015) and Nath and Dutta (2016) for the same leaf meal respectively. The differences in the proximate composition of the PLM maybe attributed to season, age, level of development of the leaves, processing methods, soil fertility and location of the study.

The haematological characteristics of growing rabbits fed diets containing pawpaw leaf meal is presented in Table 3. Packed cell volume (PCV), haemoglobin (Hb), mean cell volume (MCV), and white blood cell (WBC) differed significantly (p <0.05) across the treatment groups. Red blood cell (RBC), Mean cell haemoglobin concentration (MCHC) and mean cell haemoglobin (MCH) were statistically (P >0.05) similar across the treatments. PCV was significantly higher among the treatment groups (T_2, T_3 and T_4) in comparison with the control (T_1). T_2 also differed (p <0.05) from other treatments while T_3 and T_4 showed no significant (p >0.05) difference. The PCV range of 38.61–44.83% obtained in this study is in agreement with the normal range of 33.00–50.00% for growing rabbits as reported by Burke (1994). T_1 recorded the lowest value of 38.61% while T_2 recorded the highest value of 44.83%. The improved PCV recorded for the treatment groups may be attributed to the biologically active compounds which may have enhanced nutrient utilization or oxygen carrying capacity of the blood. However, the normal range of PCV reported in this study indicated absence or tolerable level of anti-nutrients. This further indicated that the diets were nourishing, non-toxic and influenced adequate blood supply. The haemoglobin (Hb) however, followed a similar pattern with the PCV, with T_2 having the highest and best value of 14.20 g/dl while T_1 had the lowest value. However, the improved haemoglobin concentration of rabbits on the treatment groups may imply that the dietary proteins were of high quality. Jiwuba (2018) indicated that Pawpaw leaves serve as a major source of papain, a protease used effectively as a natural digestive aid which breaks down protein. This may have enhanced the protein digestibility, availability and utilization by the experimental animals. PCV and Hb are generally used as an indicator of feed toxicity and nutritional status of animals. The values for MCV were higher (p <0.05) in T_3 and T_4 (74.60 and 75.59 fl) respectively than T_1 and T_2. The range of MCV (66.77–75.59 fl) reported in this study fell within the normal range (50 to 75 fl) for apparently healthy rabbit as reported by Burke (1994). The range is however lower than 78.97–88.97 fl reported by Jiwuba et al. (2016a) for weaner rabbits fed Gmelina arborea leaf meal, but highly comparable with range of 68.02 to 74.91

Table 3 Haematological characteristics of growing rabbits fed diets containing pawpaw leaf meal

Parameters (%)	Dietary levels (%)				
	T_1	T_2	T_3	T_4	SEM
Packed cell volume (%)	38.61^c	44.83^a	40.48^b	41.50^b	0.85
Haemoglobin (g/dl)	10.60^c	14.20^a	11.60^b	11.50^b	0.51
Red blood cell (X10$^{12/L}$)	5.34	6.71	5.42	5.49	0.21
Mean cell haemoglobin (pg)	19.80	21.16	21.40	20.95	0.23
Mean cell volume (fl)	72.30^b	66.77^c	74.60^a	75.59^a	3.65
Mean cell haemoglobin conc. (%)	27.14	31.01	28.61	27.16	1.18
White blood cell (X10$^{12/L}$)	4.50^b	8.30^a	9.50^a	8.70^a	0.73

a–c means within a row with different superscripts are significantly (P <0.05) different

Table 4 Serum biochemistry of growing rabbits fed diets containing pawpaw leaf meal

Parameters (%)	Dietary levels (%)				
	T_1	T_2	T_3	T_4	SEM
Total Protein (g/dl)	6.13^b	6.91^{ab}	6.93^{ab}	7.32^a	0.14
Creatinine (mg/dl)	1.72	1.86	1.75	1.71	0.11
AST (iu/l)	33.50	35.50	32.50	30.50	1.77
ALP (iu/l)	115.29	118.02	111.21	113.25	3.14
Sodium (mmol/L)	131.66	133.12	133.66	141.05	3.13
Potassium (mmol/L)	3.47	3.81	3.85	4.46	0.31
Chloride (mmol/l)	96.87	96.96	105.47	107.05	3.38

fl reported by Jiwuba et al. (2016b) for growing rabbits fed *Moringa oleifera* leaf meal. The differences among the treatments maybe attributed to differences in the activity of bone marrow and some haemopoietic factors influencing the capacity of bone marrow to produce red blood cells. The medicinal properties of the pawpaw leaf meal (PLM) maybe due to phytogenic compounds like chamopapain, chitinase, papain, lycopene, pectin, alkaloids (carpaine and pseudocarpaine), saponins, tannins, anthraquinones, cardiac glycosides, glucosinolate (benzylglucosinolate), choline, flavonoids (Ayoola and Adeyeye, 2010; Boshra and Tajul, 2013). These compounds have been reported (Aravind et al., 2013; Boshra and Tajul, 2013) to have antibacterial, anticoagulant, anti- rheumatoid arthritis, antiparasitic, antiviral, antifungal, anti-inflammatory, antihypertensive anti-sickling activities and promote digestive and lung health. These together with the antioxidant properties which remove free radicals from the body may have resulted to the significant improvement of the white blood cells (WBC) among the T_2, T_3 and T_4 groups in comparison with the T_1. Furthermore, the white blood cell count (WBC) ranged from 4.50 to 9.50 × X10$^{12/L}$, the values were within the range of 4.5–11 × 10 g/l reported by RAR (2009) for apparently healthy growing rabbits. These results indicated that the animals were healthy; hence decreased WBC count below the normal range (leukocytopenia) is an indication of allergic conditions and certain parasitism, while elevated values (leukocytosis) indicate the existence of a recent infection (Jiwuba et al., 2016b).

The serum biochemistry of growing rabbits fed diets containing pawpaw leaf meal (Table 4) showed no significant (P >0.05) difference across the groups, except protein. The significantly (P <0.05) higher total protein observed in T_4 in comparison with T_1 maybe attributed to presence papain which may have enhanced protein digestion and utilization among the rabbits. The non-significant (P >0.05) difference observed for creatinine, AST, ALP, sodium, potassium and chloride is an indication that the physiological status of the rabbits were not influenced by the experimental diets. Furthermore, all the parameter measured fell within the normal physiological range for apparently healthy rabbit as reported by Benson and Paul-Murphy (1999), Bradley (2001) and Putwain (2008). This further indicated that the PLM may have supported the proper functioning of the liver, maintained optimal osmolality and enhanced digestion and absorption of the minerals.

4 Conclusions

This study showed a good performance and normal physiological functioning of growing rabbits fed pawpaw leaf meal containing diets. It can be therefore, concluded that dietary supplementation of PLM in male rabbits showed no adverse effect on blood characteristics of the growing rabbits and therefore recommended for the production of healthy rabbits.

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