Tropical Animal Health and Production (2023) 55:221
https://doi.org/10.1007/s11250-023-03630-3

REGULAR ARTICLES

Effect of increasing levels of *Leucaena leucocephala* seeds to grass pellets on performance of grower rabbits

V. O. A. Ojo[1] · O. O. Adelusi[2] · O. J. Idowu[1] · O. O. Shittu[3] · F. A. Okeniyi[4] · B. M. Ogundapo[1] · F. I. Dada[5] · A. O. Oso[2]

Received: 16 November 2022 / Accepted: 9 May 2023

Abstract

The study was carried out to determine the chemical composition of composite pellets of *Megathyrsus maximus* containing *Leucaena leucocephala* seed meal at the varying level and growth responses, as well as haematological and serum biochemical of rabbits, fed the pellets for 60 days. The treatment consists of *M. maximus* and *L. leucocephala*—100:0, 90:10, 80:20, 70:30 and 60:40, respectively. Results revealed an increase ($P < 0.05$) in the proximate composition of the grass pellets with an increase in seed inclusion while there was a reduction ($P < 0.05$) in the NDF contents of the pellets. An increase in the tannin contents was recorded with an increase in seed inclusion to the grass pellets. Rabbits fed with 30 and 40% inclusions of seed in the grass pellets had similar weight gain while the lowest feed conversion ratio was recorded in rabbits fed grass with 30% seed inclusion. Packed cell volume, RBC and lymphocyte in rabbits were altered with feeding grass seed pellet ($P < 0.05$), although no particular pattern was followed. Total protein, globulin and urea contents were reduced in the rabbits as the contents of the seeds in the grass pellets increased. Albumin contents in pellets with 30% of seeds were higher in the rabbits fed the pellets above other treatments. It can be concluded that increasing the seed meal level up to 30% in grass pellets supported growth response without inflicting any side effects on the health indices of the rabbits.

Keywords Animal response · Blood profile · *Leucaena leucocephala* seed meal · *Megathyrsus maximus* · Pellets · Rabbits

Introduction

The pressing need of the livestock business is the availability of high quality and the required quantity feed for their animals (Wodajo et al. 2020). Aside from availability, the cost of purchase of the feed has skyrocketed beyond the reach of peasant farmers while it increased the cost of production to the ones that can purchase it. Both the unavailability and high cost of production have significantly affected livestock farmers as well as the decline in protein intake of people, leading to poor growth and avoidable disease conditions. To proffer solutions to the escalated cost of production of livestock, efforts should be channelled to get alternative feed resources with little or no competition with the man that are readily available, at a low cost that animals can tolerate without negatively affecting their well-being.

The animal protein requirement of the teeming population can be met by increasing rabbit production (Udeh et al. 2021). This is because the rabbit has a high fecundity rate with a high protein content that is capable of meeting the animal protein needs of people (Mukaila 2023). The short gestation interval as well as the fast growth rate of rabbits placed them ahead of other livestock in supplying protein intake of the populace (Akinola et al. 2020). Raising of rabbits in a small scale by households can ensure a steady supply of animal protein intake. This is because raising of rabbits is not complex provided good quality feeds are supplied.

V. O. A. Ojo
ojovoa@funaab.edu.ng

1 Department of Pasture and Range Management, Federal University of Agriculture, P. M. B, Abeokuta 2240, Nigeria

2 Department of Animal Nutrition, Federal University of Agriculture, P. M. B, Abeokuta 2240, Nigeria

3 Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

4 Department of Animal Science, College of Agriculture, Landmark University, PMB 1001, Omu-Aran, Kwara State, Nigeria

5 Livestock Science and Sustainable Environment, Centre for Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

Published online: 24 May 2023

For proper chewing, which is essential in the teeth trimming of rabbits, as well as aiding in breaking down the feed and for proper feed movement in the animal's gut, there is a need to supply them with sufficient fibre contents in their diets (Adji et al. 2022). Moreover, with the large ceacum they possessed, they have the potential of consuming fibrous feedstuffs (Oso et al. 2009). *Megathyrsus maximus* is an important forage plant, which is distributed across ecological niches in Nigeria and can play a major role in intensive animal production systems. However, there is a need to supplement their diets with sources of protein since feeding sole forage might not supply the maximum nutrient required for their growth performance. Since forage legumes are higher in protein content above grasses, they possess the potential to serve as cheaper feed supplementation sources for rabbits.

Browse plant seeds possess the ability to increase animal growth and productivity because of the high nutrients they contained (Arigbede et al. 2008). Effective quality can be maintained through the fortification of an animal's diet with browse plants like *Leucaena leucocephala*—a tree legume with high-quality composition contents (Ojo *et al.* 2018). The various parts of the plant contained high amounts of digestible nutrients (Albores-Moreno *et al.* 2017). The mixture of plants that contain high levels of fibre as well as the ones with high protein content will provide the nutrient required of rabbits.

To provide high-quality feed for animals, there is a need for conservation strategies that extend the shelf life of forages for year-round usage. This is particularly important for regions with a tropical climate, where wet and dry season differences cause massive fluctuations in quality and abundant forage supply (Oyaniran et al. 2018). In essence, the extensive dry season in West African countries which can last up to 6 months may have little or no influence on animal performance if curtailment strategies are put in place. Conservation such as pelletizing offers potential and additional benefits of reducing the surface area and fibre fractions, reducing feed wastage and non-selective feeding of pelleted feeds (Oyaniran et al. 2018). It is also noteworthy that the process of pelletizing could alter some of the biochemical processes in forage feedstuffs leading to quality alteration. The aforementioned authors also reported that pellets are consumed much easier when compared with forages that are long in size which will enhance feed intake and performance.

The presence of phytochemicals from the plants as a result of secondary metabolism imposed a great constraint on their use despite the high protein content in the seeds. These can, however, be resolved by the right processing methods. When introducing a new feed to animals, it is necessary to determine the effects of such feed on the health status of the animals vis-à-vis analysis of their blood parameters (Etim et al. 2014, Animashahun et al. 2022). Blood analysis indices of animals have been reported to give information on the well-being, growth performance and productivity of animals (Abo Ghanima et al. 2020).

Despite the extensive benefits of browse seeds, information about their inclusion in rabbit diet pellets is scarce. In this study, the performance of rabbits fed dietary pellets of *M. maximus* and *L. leucocephala* seed mixtures at varying levels was evaluated.

Materials and methods

Experimental site

The experiment was carried out at the Rabbit Unit of the College of Animal Science and Livestock Production Farm, the Federal University of Agriculture Abeokuta (FUNAAB), Nigeria, West Africa. The site lies within the savanna agro-ecological zone of south-western Nigeria (latitude 7°15′N, longitude 3°21′E and 348 m above sea level with an average annual rainfall of 1037 mm). Mean annual temperature and humidity are 30.5 °C and 82%, respectively (Earth.google.com/).

Collection of *Leucaena leucocephala seed and Megathyrsus maximus*

Matured pods of *Leucaena leucocephala* were collected from the multipurpose tree arboretum of the Department of Pasture and Range Management (PRM), FUNAAB. The content of the pod was removed by threshing and was cured in the sun for 3 days. The seeds were treated by boiling 500 g in 1 l of water on a hot plate (Stuart, heat stir, CB162, UK) with four replicates (100 °C for 15 min), and the seeds were dried and milled using a laboratory hammer mill (Model DFZH-Bühler, Uzwil, Switzerland) sieved with a 3 mm screen. The chemical composition of *Leucaena leucocephala* seeds revealed that they contained dry matter 95%, crude protein 32%, ether extract 7%, ash 4.5% and tannin 1.3%.

Megathyrsus maximus grass was sourced from an established plot which had previously been sown with *M. maximus* 2 years prior to the conduct of this experiment and fertilised annually with 5.0 t/ha of poultry manure (approximately 150 kg N/ha applied in split dosage).

The grasses on the plots were cut to 15 cm above ground level during the early rainy season (immediately after the first rain) and allowed a re-growth period of 6 weeks. The grasses were harvested in the 6th week from 15 cm above ground level. The harvested grasses were chopped to about 5 cm lengths, dried and milled.

Procedure for pelletizing

The milled grass was mixed with the milled seeds of *L. leucocephala* on dry matter weight in accordance with the treatments (separate mixture for each proportion—100:0, 90:10, 80:20, 70:30 and 60:40). The composite samples were homogenised using a mixer, and a binder (cassava flour) of 1 kg was homogenised with 100 kg of the sample, and water was used to moisten the emerging composite mixture. The composite mixture was pelletized using a pelletizing machine RD-Pellet Machine (Model: DE-150, Manufacturer: Dezhou Runde Metal Products Co., Ltd.) fitted with a 6 mm die size. The pelletizer produced a pellet with an average size of 40 mm. The resulting pellets were spread out to dry. The pellets were put in polyethene bags for proper storage at room temperature.

Experimental design

The experiment was laid out as a completely randomised design with 5 treatments replicated 6 times.

The treatments were as shown below:

100% M. maximus + 0% L. leucocephala seeds pellet
90% *M. maximus* + 10% *L. leucocephala* seeds pellet
80% *M. maximus* + 20% *L. leucocephala* seeds pellet
70% *M. maximus* + 30% *L. leucocephala* seeds pellet
60% *M. maximus* + 40% *L. leucocephala* seeds pellet

Experimental animals and management

Thirty New Zealand white grower bucks (average bodyweight of 600 ± 16 g) at 6 weeks old were used in this study for a period of 8 weeks. The animals were divided into five treatments of *M. maximus* and *L. leucocephala* seeds—100:0, 90:10, 80:20, 70:30 and 60:40, respectively, with 6 animals per treatment. Two weeks before the rabbits were brought in, the hutch and environment were swept, washed and disinfected. When the rabbits were brought to the farm, they were administered with antibiotics and dewormer. The rabbits were put in an individual hutch with a dimension of 60 × 60 × 50 cm for each animal. Initial weights of the rabbits were noted and were stratified to the experimental diets. Feeders and drinkers that were made of concrete were provided in each of the hutches for the experimental animals. Based on the treatments, 100 g of each of the experimental pellets and a commercial pellet of 50 g were mixed thoroughly together before feeding to rabbits in the same concrete feeders. This was done to prevent rabbits from selecting the individual pellets in the mixtures. Good ventilation was ensured for the rabbits throughout the period of the study. Feeders and drinkers were washed and cleaned every morning with daily provision of feed and drinking water.

A commercial rabbit pellet feed with the nutrient composition listed in Table 1 was purchased from a reputable agro-allied venture and fed alongside the composite experimental pellets. This was done to ensure the minimal nutrient requirement of the rabbits was met and to improve the intake of the experimental diet (Iyeghe-Erakpotobor *et al.* 2006).

Table 1 Chemical composition of experimental pellets fed (*M. maximus* and *L. leucocephala* seeds) and commercial concentrate diet used in this study

Parameters (g/kg)	*M. maximus* : *L. leucocephala*					SEM	*P* value	Commercial diet
	100:00	90:10	80:20	70:30	60:40			
Dry matter	944.30^{c}	943.60^{d}	945.70^{a}	944.70^{b}	939.70^{e}	0.55	0.000	950.00
Ether extract	39.67^{e}	46.90^{c}	42.50^{d}	50.80^{b}	95.40a	5.49	0.000	50.00
Crude protein	82.00^{e}	105.00^{d}	113.30^{c}	133.40^{b}	159.50^{a}	7.02	0.000	175.00
Ash	70.00^{e}	95.10^{d}	103.50^{c}	111.60^{b}	119.20^{a}	4.54	0.000	60.00
Organic matter	930.00^{a}	904.90^{b}	896.50^{c}	888.40^{d}	880.80^{e}	4.54	0.000	940.00
Nitrogen free extract	136.81^{a}	85.80^{c}	92.60^{b}	82.60^{c}	31.70^{d}	8.95	0.000	340.00
Neutral detergent fibre	671.52^{a}	667.20^{b}	648.10^{c}	621.60^{d}	594.20^{e}	7.78	0.000	375.00
Acid detergent fibre	322.44^{e}	363.90^{a}	347.60^{b}	336.00^{c}	324.80^{d}	4.10	0.000	190.00
Acid detergent lignin	50.93^{a}	48.00^{b}	44.30^{c}	40.93^{d}	38.20^{e}	1.23	0.000	57.20
Hemicellulose	349.08^{a}	303.30^{b}	300.50^{c}	285.60^{d}	269.40^{e}	7.12	0.000	185.00
Cellulose	271.51^{e}	315.90^{a}	303.30^{b}	295.07^{c}	286.60^{d}	4.01	0.000	132.80
Lignin/Cellulose	0.19^{a}	0.15^{b}	0.15^{b}	0.14^{b}	0.13^{b}	0.01	0.042	0.43
Tannin (mg kg^{-1} DM)	ND	8.40^{b}	9.00^{b}	9.20^{b}	13.80^{a}	1.21	0.000	ND
Digestible energy kcal/kg	1.81^{a}	1.57^{b}	1.59^{b}	1.58^{b}	1.57^{b}	0.02	0.000	2.56

$^{a–e}$means in the same row with different superscripts are significantly ($P < 0.05$) different

SEM, standard error of mean; Digestible energy was estimated using De Blas et al. (2020) formula

Feed intake and weight gain measurements

During the period of the study, the feed intake was determined by weighing the diets offered to each of the rabbits and the leftovers every day. After the initial weight of the animals was recorded, they were then measured every week to compute for weight change as influenced by the dietary treatments. The feed conversion ratio was the quantity of feed consumed (g/day) by weight change (g/day) in the rabbits.

Blood sampling

At the end of the experiment, a sample of blood was taken from the ear marginal vein of the rabbits to analyse the blood. Sample bottles with ethylene-diamine-tetraacetic acid in them were used to collect blood for haematological parameters while samples for serum biochemical determination were put into plain test tubes for analysis.

Chemical analyses

Samples of experimental pellets and the faeces output were dried at 65 °C for dry matter contents. The samples were thereafter milled through a 1 mm sieve using DFZH-Bühler, Uzwil, Switzerland hammer mill. These were analysed for the proximate composition according to AOAC (2000) and fibre fractions according to Van Soest et al. (1991) procedure. Hemicellulose and cellulose contents were calculated. Tannin content was determined according to the procedure of Hayat et al. (2020). Organic matter was calculated as 1000 – ash (g kg^{-1}) (Lanyasunya et al. 2007) while digestible energy (DE) was calculated as DE = GE (gross energy) × (0.867 – 0.0012ADF) (De Blas et al. 2020).

Haemoglobin (Hb), red blood cell (RBC), packed cell volume (PCV) and white blood cell (WBC) were determined according to the procedure of Mitruka and Rawnsley (1981). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were estimated by calculation using standard formulae by Jain (1986). Glucose was determined using a spectrophotometric procedure as described by Barham and Trinder (1972), while total serum protein, albumin and globulin were determined using the bromocresol purple method (Ueno *et al.* 2013).

Economics of production of rabbits fed *M. maximus* and *L. leucocephala* seed pellets at varying levels

The prevailing market price of the feed ingredient at the time of the study was used to calculate the cost of the feed consumed and the cost of feed per kilogramme (kg) live weight gain.

Statistical analysis

Data obtained were analysed as a completely randomised design and subjected to a one-way analysis of variance of the SPSS (IBM SPSS Statistics 23) software. The means were compared using Duncan's multiple range test to identify differences between means, and significant differences were declared if $P < 0.05$.

Results

Chemical composition of *M. maximus* and *L. leucocephala* seed pellets at varying levels

The result of the chemical composition of *M. maximus* sole and its mixtures with *L. leucocephala* seed pellets at varying levels are shown in Table 1. Results revealed an increase ($P < 0.05$) in CP and ash contents of grass pellets with the increase in the seed inclusions while there was a reduction ($P < 0.05$) in the NDF contents of the pellets. An increase in the tannin contents was recorded with an increase in seed inclusion to the grass pellets. The ratio of ADL and cellulose as well as the digestible energy of the feed were higher ($P < 0.05$) in sole grass pellets above treatments with seed inclusion.

Growth parameters of rabbits fed *M. maximus* and *L. leucocephala* seed pellets at varying levels

The growth parameters and the nutrient intake of rabbits fed *M. maximus* and *L. leucocephala* seed-based pellets at varying levels are presented in Table 2. Rabbits fed with 30 and 40% inclusions of seed in the grass pellets had similar weight gain while the lowest feed conversion ratio was recorded for rabbits fed grass pellets with 30% seed inclusion. Rabbits increased in weight gain with an increase in levels of seed inclusion in grass pellets. Rabbits fed with sole grass pellets had the highest ($P < 0.05$) feed conversion ratio above other treatments. All values obtained for daily and total feed intakes of rabbits were statistically ($P > 0.05$) similar despite varying levels of the seed in the pellets. Similarly, no difference ($P > 0.05$) in cost incurred during production was recorded for all the treatments investigated in this study.

Haematological parameters of rabbits fed varying levels of *M. maximus* and *L. leucocephala* seed pellets

Effects of haematological parameters on the feeding of *L. leucocephala* seed in grass pellets at varying levels to rabbits are shown in Table 3. The parameters were significantly

Table 2 Performance traits of rabbits fed *M. maximus* and *L. leucocephala* seed based pellets at varying levels

Performance traits		*M. maximus : L. leucocephala*				SEM	*P* value
	100:0	90:10	80:20	70:30	60:40		
Initial weight (g)	595.85	617.76	586.18	613.33	587.68	14.29	0.955
Final weight (g)	910.00[d]	1134.60[bc]	1080.12[c]	1273.33[a]	1249.48[ab]	37.88	0.000
Weight gain (g)	314.15[b]	516.84[ab]	493.94[ab]	660.00[a]	661.80[a]	43.01	0.027
Daily weight gain (g)	5.24[b]	8.61[ab]	8.23[ab]	11.00[a]	11.03[a]	0.72	0.027
Total Feed Intake (g/day)	3372.11	4332.40	4557.42	4328.70	4526.76	192.54	0.289
Daily feed intake (g/day)	56.20	72.21	75.96	72.15	75.45	0.00	0.289
Cost/kg of feed (₦)	606.98	787.70	838.64	805.86	850.87	36.91	0.218
Feed cost/weight gain ($/kg)	4.83	4.09	4.39	3.15	3.29	0.28	0.271
Feed conversion ratio	10.58[a]	9.03[c]	9.54[b]	6.78[e]	7.00[d]	0.39	0.000

[a–e]means in the same row with different superscripts are significantly ($P < 0.05$) different

SEM, standard error of the mean, current naira (N) to dollar ($) exchange ratio is ₦400 = $1

Table 3 Haematological parameters of rabbits fed varying levels of *M. maximus* and *L. leucocephala* seed pellets

Parameters	Normal range	*M. maximus* × *L. leucocephala*					SEM	*P* value
		100:0	90:10	80:20	70:30	60:40		
Packed cell volume (%)	36–43	42.500[a]	36.50[b]	41.50[a]	37.50[b]	34.50[b]	0.89	0.001
Haemoglobin (g/dl)	10–17	12.15[bc]	12.15[bc]	13.85[a]	12.60[b]	11.50[c]	0.24	0.004
Red blood cell (x10^{12} /L)	5–8	7.00[a]	6.05[b]	7.05[a]	6.30[b]	5.70[b]	0.16	0.002
White blood cell (x10^9/L)	5.2–12.5	7.30[b]	11.50[a]	11.65[a]	10.30[a]	10.45[a]	0.46	0.001
Neutrophils (%)		33.20[a]	28.00[c]	32.50[ab]	29.50[bc]	27.50[c]	0.72	0.005
Lymphocyte (%)	30–85	64.00[bc]	68.50[a]	63.50[c]	67.50[ab]	70.00[a]	0.81	0.009
Eosinophils (%)	1–4	1.500[a]	0.00[c]	0.00[c]	1.00[ab]	0.50[bc]	0.19	0.018
Basophils (%)	1–7	1.26[b]	2.00[ab]	2.50[a]	1.50[ab]	2.00[ab]	0.17	0.176
Monocyte (%)	1–4	0.73[b]	1.50[a]	1.50[a]	0.50[b]	0.00[b]	0.179	0.004
Mean corpuscular volume (fl)	58–673	60.73[a]	60.33[ab]	58.89[b]	59.54[ab]	60.58[a]	0.23	0.067
Mean corpuscular haemoglobin (pg)		17.36[c]	20.08[a]	19.65[b]	20.01[ab]	20.20[a]	0.29	0.000
Mean corpuscular haemoglobin concentration (g/dl)	29–37	28.59[c]	33.28[b]	33.37[ab]	33.60[a]	33.34[ab]	0.52	0.000

[a–e]means in the same row with different superscripts are significantly ($P < 0.05$) different

SEM, standard error of the mean

affected ($P < 0.05$) with the mixtures of *L. leucocephala* seed with grass pellets in the rabbits fed. The PCV, HB, RBC and NEUT ranged from 34.50 to 42.50%, 11.50 to 13.85 g/dl, 5.70 to 7.05 × 10^{12}/L and 27.50 to 33.20%, respectively. All the rabbits fed pellets with seed inclusion had higher WBC concentrations when compared with the control group.

Serum biochemical parameters of rabbits fed varying levels of *M. maximus* and *L. leucocephala* seed pellets

Serum biochemical parameters of rabbits fed *M. maximus* and *L. leucocephala* seed pellets at different proportions are shown in Table 4. Total protein, globulin, asparatate aminotransferate and urea contents reduced in the rabbits as the contents of the seeds in the grass pellets increased. Albumin contents in pellets with 30% of seeds were higher in the rabbits fed the diets above other treatments.

Discussion

The increased CP content in the grass pellet following the inclusion of seeds at increasing levels implies that the seeds have the ability to enrich the quality of the grass. This agreed with the past finding that high-nitrogen multipurpose trees can be used as supplements to increase the quality of tropical grasses (Babayemi and Bamikole 2006a). The potential of the seeds is that it has the ability to augment the nutrients lacking in the grass, especially CP content. Since tropical browse seeds are easy to come by, they can be used in place of expensive as well as scarce ingredients like groundnut

Table 4 Serum biochemical parameters of rabbits fed varying levels of *M. maximus* and *L. leucocephala* seed pellets

Parameters		*M. maximus* × *L. leucocephala*					SEM	*P* value
	Normal range	100:0	90:10	80:20	70:30	60:40		
Total protein (g/dl)	5.4–7.5	5.90^{b}	7.05^{a}	6.65^{a}	6.45^{ab}	5.75^{b}	0.15	0.008
Albumin (g/dl)	2.7–5.0	2.25^{bc}	3.50^{bc}	3.50^{bc}	4.30^{a}	3.10^{c}	0.11	0.809
Globulin (g/dl)	1.5–2.7	2.25^{bc}	3.55^{a}	3.20^{a}	2.10^{c}	2.65^{b}	0.16	0.000
Asparatate aminotransferate (U/l)	35–130	47.50^{c}	83.00^{a}	73.50^{b}	72.00^{b}	68.50^{b}	3.23	0.000
Alanine aminotransferate (U/l)	45–80	40.00^{c}	55.00^{a}	56.50^{a}	53.00^{ab}	51.00^{b}	1.62	0.000
Glucose (mg/dL)	75–155	73.65^{b}	103.40^{a}	84.85^{ab}	98.85^{a}	91.00^{ab}	3.88	0.088
Urea (mol/l)	20–45	15.25^{b}	18.10^{ab}	18.40^{a}	17.45^{ab}	16.35^{ab}	0.46	0.154
Total bilirubin (mg/dl)		0.30^{c}	0.95^{b}	0.35^{bc}	1.30^{a}	1.10^{ab}	0.11	0.000
Direct bilirubin (mg/dl)	0–0.7	0.12^{c}	0.65^{d}	0.35^{bc}	0.35^{bc}	0.50^{ab}	0.06	0.013
Creatinine (mg/dl)	0.5–2.5	1.23^{a}	0.70^{b}	0.50^{b}	0.85^{ab}	0.40^{b}	0.10	0.020

$^{a–e}$means in the same row with different superscripts are significantly ($P < 0.05$) different

SEM, standard error of the mean

cake, cotton seed cake and soybean seeds used by livestock owners in Nigeria, which will increase the cost of production.

Higher CP values above 10% were recorded in grass pellets with seed inclusion which fall in line with recommendations for growth/maintenance in animals (Oyewole and Aderinola 2019). Aside from CP contents, there was an increase in EE, ash and tannin contents of the pellets with an increase in seed inclusion, probably because of the positive contribution of *L. leucocephala* seeds to the diet. This study showed that the inclusion of *L. leucocephala* seeds significantly improved the quality of the grass pellets. Patra and Saxena (2011) reported that plant tannins can improve feed efficiency and animal health, and Mahanani et al. (2020) also reported that tannin content in *L. leucocephala* contains the potential to reduce CH_4 emissions and improve feed protein utilization. However, increases in compounds such as tannin may have a deleterious effect on the target animals that the diet is being fed; tannin values from this study were lower than the range between 20 and 50 g kg^{-1} and as a result it will not influence the microorganism in animals negatively (Nath et al. 2022). Increasing the inclusion of *L. leucocephala* seeds in the pellets also caused a decline in the fibre content. This was in consonance with the report of Ojo et al. (2018) with similar findings when browse seeds were ensiled with tropical grasses.

Increased weight gain obtained for rabbits fed with 30 and 40% inclusions of seed in the grass pellets compared with a diet without seed inclusion (control), indicated that the pellets were well utilized as a result of its high quality brought about by the addition of seeds. This agreed with the reports of Babayemi et al. (2004a, b) that higher nutrients brought about by mixing of grass and legume together make them a diet suitable to supply needed nutrients to animals for their expected performance and profit for livestock farmers (Babayemi and Bamikole 2006b). A decrease in the body weight of animals has been associated with a decline in protein inclusion in feed (Retnaningrum et al. 2021) and a gain of animal decline with a reduction in protein in the feed, which was true in this study. In their study, Tokofai et al. (2021) reported increased weight gain and lower FCR at all levels in a broiler-fed diet with the inclusion of *Vernonia amygdalina* leaf meal. Higher weight gain of rabbits was recorded in this study when compared with the range of 87.5–281.2 g in rabbits fed graded levels of soybean cheese waste/maize offal diet and Brachiaria grass hay (Iyeghe-Erakpotobor et al. 2006). The beneficial function of *L. leucocephala* seed was revealed in the tannin protein-binding capacity with an increase in the weight of the rabbits with seed inclusion reported in this study (Mahanani et al. 2020). This study revealed that the quality as well as the type of forage given to rabbits have corresponding effects on their growth.

Omoikhoje et al. (2006) reported that better growth was recorded in animals fed concentrates and supplemented with forages than sole concentrate diet only. In the present study, the inclusion of up to 40% of seeds in grass pellets promoted weight gain in rabbits when fed along with the commercial pellets. This agreed with the report of Abubakar et al. (2011) that there were no side effects on the growth performances of weaned rabbits fed up to 45% level of *Moringa oleifera* leaf meal in their diets. In order to have more profit by farmers with minimal inputs in terms of ingredients in feed formulation, feeding of forages along with concentrate diets to animals will go a long way to achieve this purpose with reduced cost of production at the end (Aderinboye et al. 2015). The increase in weight gain of the rabbits with increased seed inclusion might be as a result of sufficient nutrient intake, digestibility and absorption due to moderate fibre content in the feeds.

Rabbits fed with 40% inclusions of seed in the grass pellets were the most expensive among all other treatments although not statistically significant. The feed cost/weight gain for rabbits fed 30% and 40% of seed in grass pellets were lower compared to the rest treatments. This could be the result of increasing seeds in the feed-based mixture. The best feed conversion ratio obtained for rabbits fed with 30% inclusions of seed in the grass pellets implied an improved conversion of feed to life weight gain. This was followed by rabbits fed with 40% inclusions of seed in the grass pellets. The relative feed cost/weight gain for rabbits with higher inclusion of seeds compared to others could be an indication that it is economical for a modest production of rabbits.

The feed intake seems to increase with an increase in the seed inclusion of up to 20% of seed in the grass pellet-based diet, but reduced at 30% of seed inclusion. This might be a result of increasing the level of the seeds which elevates the resultant dietary CP level and, hence, stimulates increased feed intake. A reduction in feed intake of 228.72 g could be a result of tannin interphase in the *L. leucocephala* seeds which picked up again at 40% of seed in the grass pellet. The slight increase in feed intake as the inclusion of the seeds increased from 10 to 20% in the grass pellets might be due to the increased CP content of the diet as the seed increased.

Analysis of the haematology parameters of animals that are fed the novel non-conventional feed ingredients is necessary so as to assess their suitability and quality as animal diets (Attia *et al*. 2018). The haematological values in rabbits that were fed grass pellets with varying levels of *L. leucocephala* seeds are indicative of better nutrient utilization, absorption and assimilation into the blood circulatory system of the rabbits (Onu 2010). Most of the values of haematology in this study are within the recommended range for healthy animals (Ogbuewu *et al.* 2010). The white blood cell (WBC) count in rabbits fed the pellets with and without seeds fell within the acceptable range for healthy rabbits which indicated that the feed conferred immunity to diseases on the animals. The increase in WBC obtained for rabbits fed the experimental diets containing seed when compared with the control group could be the result of increasing tannin content as the inclusion of seed increased. Increased seed tannin content may be caused the resultant WBC to increase. However, the dietary tannin content in the present study was still within the tolerable levels considered non-injurious to animals. Moreso, the values of WBC in this study fall within the recommended range for healthy rabbits.

The RBC reported generally for rabbits in this study suggests a better carrying capacity of oxygen to the tissues at pressures sufficient to permit its rapid diffusion. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were indices of red blood cells measured on complete blood count. The MCH is the haemoglobin per red blood cell while MCHC is the average concentration of haemoglobin in red blood cells. This indicates whether or not an animal's red blood cell has more or less haemoglobin than usual. The range of MCH and MCHC in rabbits under study indicated that the rabbits were in good health and their blood level conditions functioned normally as reported by Research Animal Resources [RAR] (2009) that normal MCHC values for rabbits should range from 27 to 37 g/dl.

Reduced haemoglobin concentration in rabbits fed grass pellets with 40% seed inclusion when compared with the ones fed with pellets containing 30% seed inclusion implied poor dietary protein utilization. And this might be due to a higher level of antinutritional contained in pellets with 40% seed inclusion interfering with dietary protein utilization.

Protein content in a diet has been reported to have effects on blood total protein and its components (Obikaonu et al. 2012). The reduction in the total protein and globulin of rabbits fed with 40% seed in grass pellet inclusion, when compared with a pellet of 10% seed inclusion, could be as a result of the antinutrients contents in the seed included into the pellets (Odetola et al. 2012). In their study, Obikaonu et al. (2012) and Tijani et al. (2015) observed a decrease in serum protein with Neem and Moringa leaves, respectively, into broiler diets. This was similar to the present study. The value of total protein in this study was within the normal range of 5 to 8 g/dl (Gasmalbari et al. 2020). This revealed that protein metabolism by the animal has not been altered because the synthesis of protein is related to the quantity available in the diet (Coelho-Junior et al. 2020). The results in serum glucose in this study revealed that bioactive contents in *L. leucocephala* seeds were not high enough to prevent the metabolic pathway of energy (Kunugi and Mohammed Ali 2019) given the inclusion levels of *L. leucocephala* seeds utilized in this study. This suggested that even at 40% inclusion of *L. leucocephala*, the rabbits will still be able to meet their nutrient requirements. Higher blood globulin values recorded for rabbits with lower contents of seed (10 and 20%) in grass pellets compared with the ones with higher seed (30 and 40%) in grass pellets might be due to lower CP contents in the pellets with lower seed contents. Globulin contents in animals' blood help the animals to fight diseases as the result of improvement in the immune system of the body (Iheukwumere et al. 2005). The urea content in all the treatments was lower than the normal range expected of normal rabbits. The reason for this cannot be ascertained. However, no negative response was exhibited by the animals during the study, indicating that the pellets were safe as feed for rabbits. This study revealed that incorporating *L. leucocephala* seeds into rabbits' grass pellets enhanced their blood parameters.

From this study, it could be inferred that *L. leucocephala* seeds can be mixed with *M. maximus* grass in rabbits' pellets to 30% for better growth and utilization without hampering the health of the animal. The use of grass and *L. leucocephala* seeds in the formulation of rabbit feed is recommended since it is less competitive and readily available.

Author contributions All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by V. O, O. *A., O. I., O. S., F. O., B. O., F. D and A. O.* The manuscript writing and review were contributed to by all authors.

Funding The present research study was not funded by any special funding agencies. All expenses were paid for by all the researchers.

Data availability All data associated with this research were indicated and used in the manuscript submitted.

Code availability There is no custom code associated with all data used in this study. However, associated data are available upon request.

Declarations

Ethics approval This work was approved and carried out in accordance with the guidelines of the Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Nigeria.

Consent to participate All authors included in this manuscript gave their consent to participate in the research field experimentation, data collection and manuscript preparation.

Consent for publication All authors consented to the publication of this manuscript.

Conflict of interest The authors declare no competing interests.

References

Abo Ghanima, M.M., Elsadek, M.F., Taha, A.E., Abd El-Hack, M.E., Alagawany, M., Ahmed, B.M., Elshafie, M.M. and El-Sabrout, K., 2020. Effect of housing system and rosemary and cinnamon essential oils on layers performance, egg quality, haematological traits, blood chemistry, immunity, and antioxidant. *Animals*, 10(2),.245.

Abubakar, M., Yusuf, A. U., Dome, U. D., Ibrahim, U. and Muhammad, A. S. 2011. Growth performance, carcass and organ characteristics of growing rabbits fed graded levels of *Moring oleifera* leaf meal diets. Proceedings of the 16th Annual Conference of Animal Science Association of Nigeria (ASAN) , 365–368.

Aderinboye R. Y., Oladeji O. T., Abaire M. A., Sobayo, R. A., Oso, A. O., Oni A. O., Yusuf K. O., Osho S. O. and Bamgbose A. M. 2015. Performance of weaner rabbits fed a concentrate diet supplemented with pawpaw leaves. *Tropical Animal Health Production,* 47:323–329 DOI https://doi.org/10.1007/s11250-014-0723-1

Akinola, O.S., Irekhore, O.T. and Ademolue, R.O., 2020. Evaluation of growth, reproductive performance and economic benefits of rabbits fed diets supplemented with turmeric (*Curcuma longa*) powder. *Egyptian Poultry Science Journal*, 40(3), 701-714.

Adji, A.V., Pedersen, A.Ø. and Agyekum, A.K., 2022. Obesity in pet rabbits: A narrative review. *Journal of Exotic Pet Medicine*

Albores-Moreno, S., Alayón-Gamboa, J.A., Ayala-Burgos, A.J., Solorio-Sánchez, F.J., Aguilar Pérez, C.F., Olivera-Castillo, L. and Ku-Vera, J.C. 2017. Effects of feeding ground pods of *Enterolobium cyclocarpum* Jacq. Griseb on dry matter intake, rumen fermentation, and enteric methane production by Pelibuey sheep fed tropical grass. *Tropical Animal Health Production,* 49, 857–866. https://doi.org/10.1007/s11250-017-1275-y

Animashahun, R. A., Alabi, O. O., Okeniyi, F. A., Olawoye, S. O., Shoyombo, J. A. and Falana, B. M. 2022. Performance and blood profile of Noiler chickens fed diets containing graded levels of *Parkia biglobosa* leaf meal. *Food Research*, 6(5), 256-265.

AOAC (2000). Official methods of analysis 15, Association of Official Analytical ChemistsWashington D.C

Arigbede, O. M., Anele, U., Jolaosho, A. O., Olanite, J. A., Onifade, J. S. and Waheb, T. A. 2008. Chemical composition and *in vitro* gas production of African Bread fruit (*Treculia africana*) var Decne. *Achivos de Zootecnia* 58(28): 113-121.

Attia, Y.A., Bakhashwain, A.A. and Bertu, N. K. 2018. Utilisation of thyme powder (*Thyme vulgaris* l.) as a growth promoter alternative to antibiotics for broiler chickens raised in a hot climate. *European Poultry Science*, 82, 238. https://doi.org/10.1399/eps.2018.238

Babayemi, O.J. and Bamikole, M.A. 2006a. Effects of *Tephrosia candida* DC leaf and its mixtures with Guinea grass on *in vitro* fermentation changes as feed for ruminants in Nigeria. *Pakistan Journal of Nutrition*, 5: 14-18.

Babayemi, O.J. and Bamikole, M.A. 2006b Nutritive value of *Tephrosia candida* seed in West African dwarf goats.Journal of Central European Agriculture, 7: 731-738

Babayemi, O.J., Demeyer, D. and Fievez, V. 2004a. Nutritive value and qualitative assessment of secondary compounds in seeds of eight tropical browse, shrub and pulse legumes. *Communications in Agricultural and Applied Biological Sciences,* 69: 103-110

Babayemi, O. J., Demeyer, D. and Fievez, V. 2004b. In vitro rumen fermentation of tropical browse seeds in relation to their content of secondary metabolites. Journal of Animal and Feed Sciences, 13, 31-34.

Barham, D. and Trinder, P. 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst*, 97(1151), 142-145.

Coelho-Junior, H.J., Marzetti, E., Picca, A., Cesari, M., Uchida, M.C. and Calvani, R., 2020. Protein intake and frailty: a matter of quantity, quality, and timing. Nutrients, 12(10), 2915.

Etim, N. N., Williams, M. E., Akpabio, U. and Offiong, E. E. A. 2014. Haematological parameters and factors affecting their values. *Agricultural Science,* 2 (1), 37-47.

De Blas, C. and Mateos, G. G. 2020. 12 Feed Formulation. Nutrition of the Rabbit, 243.

Gasmalbari, E., Awad, Y. and Abbadi, O., 2020. Biochemical, Haematological and Histopathological Changes caused by *Senna obtusifolia* (L) in Albino Rats*International Journal of Recent Research in Life Sciences,* 7(3)45-50).

Hayat, J., Akodad, M., Moumen, A., Baghour, M., Skalli, A., Ezrari, S. and Belmalha, S., 2020. Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. *Heliyon*, *6*(11), e05609.

Iheukwumere, F. C., Okoli, I. C., Anyanwu, G. A. and Esonu, B. O. 2005. Growth performance, haematology and serum biochemical constituents of grower rabbits fed *Microdesmis puberula, Hook-Euphorbiaceae. Animal Production Research Advances*, 1, 24-31. https://doi.org/10.4314/apra.v1i1.36287

Iyeghe-Erakpotobor G. T., Osuhor C.U and Olugbemi T. S. 2006. Performance and digestibility of weaner rabbits fed graded levels of soybean cheese waste/maize offal diet and Brachiaria grass hay. *African Journal of Biotechnology,* 5 (17), 1579-1583.

Jain, N. C. 1986. Schalman's Veterinary Haematology, 4th. Lea and Babings, Philadelphia, 208–224

Kunugi, H. and Mohammed Ali, A., 2019. Royal jelly and its components promote healthy aging and longevity: from animal models to humans. *International Journal of Molecular Sciences*, 20(19), 4662.

Lanyasunya, T.P., Wang, H.R., Kariuki, S.T., Kuria, D.M., Chek, A.L. and Mukisira, E.A., 2007. Effect of maturity on the mineral content of hairy vetch (Vicia villosa). Tropical and Subtropical Agroecosystems, 7(1), pp.53-58.

Mahanani, M.M.P., Kurniawati, A., Hanim, C., Anas, M.A. and Yusiati, L.M., 2020. Effect of (*Leucaena leucocephala*) leaves as tannin source on rumen microbial enzyme activities and *in vitro* gas production kinetics. In *IOP Conference Series: Earth and Environmental Science* (Vol. 478, 012088 IOP Publishing.

Mitruka, B.M. and Rawnsley, H.M. 1981. Clinical Biochemical and Hematological Values in Normal Experimental Animals and Normal Humans. Madison Publishing, New York, New York, USA

Mukaila, R., 2023. Measuring the economic performance of small-scale rabbit production agribusiness enterprises. *World Rabbit Science*, *31*(1), pp.35-46.

Nath, H., Samtiya, M. and Dhewa, T., 2022. Beneficial attributes and adverse effects of major plant-based foods anti-nutrients on health: A review *Human Nutrition & Metabolism*200147. https://doi.org/10.1016/j.hnm.2022.200147

Obikaonu, H.O., Okoli, I.C., Opara, M.N., Okoro, V.M.O., Ogbuewu, I.P., Etuk, E.B. and Udedibie, A.B.I. 2012. Haematological and serum biochemical indices of starter broilers fed leaf meal of Neem (*Azadirachta indica*). *Journal of Agricultural Technology,* 8(1), 71–79.

Odetola, O.M., Adetola, O.O., Ijadunola, T.I., Adedeji, O.Y. and Adu, O.A. 2012. Utilization of Moringa (*Moringa oleifera*) leaf meal as a replacement for soya bean meal in rabbit's diets. *Scholarly Journal of Agricultural Science*, 2(12), 309–313.

Ogbuewu, I. P., Uchegbu, M. C., Okoli, I. C. and Iloeje, M. U. 2010. Toxicological effects of leaf meal of ethnomedicinal plant - neem - on serum biochemistry of crossbred New Zealand white typed rabbit bucks. Report and Opinion, 2 (2): 54-57

Ojo, V.O.A., Akinade, G.A., Fasae, O.A. Akinlolu, A.O. 2018. Effect of treatment methods on the nutritive quality of elephant-ear seeds (*Enterolobium cyclocarpum* Jacq Griseb) as feed for ruminant production. *Pertanika Journal Tropical Agricultural Science,* 41: 453–462.

Omoikhoje, S. O., Bamgbose, A. M., Aruna, M. B. and Animashahun, R. A. 2006. Response of weaner rabbits to concentrate supplemented with varying levels of *Syndrella nodiflora* forage. *Pakistan Journal of Nutrition*, 5 (6), 577–579.

Onu, P.N. (2010). Evaluation of two herbal spices as feed additives for finisher broilers. *Biotechnology in Animal Husbandry*, 26(5–6), 383–392. https://doi.org/10.2298/bah1006383o

Oso AO., Fafiolu AO., Dele PA., Ojo V.O.A., Yusuf KA. Amole TA, Oni OO. and Odunfa OB. 2009. Growth response and nutrient digestibility of weaner rabbits fed macaroni waste. Proceedings of 14th Annual Conference of Animal Science Association of Nigeria. LAUTECH Ogbomoso, Nigeria, pp 493 – 495.

Oyaniran, D.K., Ojo, V.O.A., Aderinboye, R.Y., Bakare, B.A. and Olanite, J.A. 2018. Effect of pelleting on nutritive quality of forage legumes. *Livestock Research for Rural Development,* 3075

Oyewole, S. and Aderinola, O., 2019. Growth Performance and Bio-Economic Indices of Varying Mixtures of Grass-Legume Pellets Fed to West African Dwarf (WAD) Goats. *Journal of Biology, Agriculture and Healthcare,* 9(24).

Patra, A.K. and Saxena, J., 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture*, *91*(1), pp.24-37.

Research Animal Resource [RAR]. 2009. Reference values for laboratory animals: Normal haematological values. RAR Websites, RAR, University of Minnesota. Retrieved from http://www.ahc.umn.edu/rar/refvalues.html.

Retnaningrum, S., Harper, K.J. and Poppi, D.P., 2021. Formulating rations with cassava meal to promote high live weight gain in crossbred Limousin bulls. *Animal*, *15*(2), p.100125.

Tijani, L.A., Akanji, A.M., Agbalaya, K. and Onigemo, M. 2015. Haematological and serum biochemical profiles of broiler chickens fed diets containing Moringa leaf meals. *Agro-Science Journal of Tropical Agriculture, Food, Environment and Extension,* 14(3), 7–11. https://doi.org/10.4314/as.v14i3.2

Tokofai, M.B., Idoh, K., Oke, E.O. and Agbonon, A. (2021). Growth performance, haematological and biochemical parameters in broilers fed diets with varying levels of *Vernonia amygdalina* leaf meal. *European Poultry Science,* 84, 321. https://doi.org/10.1399/eps.2020.321

Udeh, N.P., Okeke, J.J., Okeke, O.P., Obudulu, C. and Okafor, K.P., 2021. Comparative studies of growth performance of Rabbits (*Ornyctolagus cunniculus*) fed on rabbit feeds and diverse foodstuff in captivity. *Asian Journal of Research in Zoology*, *4*(3), pp.9-17.

Ueno K., Naganos S., Hirano Y., Horiuchi J. and Saitoe M. 2013. Long-term enhancement of synaptic transmission between antennal lobe and mushroom body in cultured Drosophila brain. *Journal of Physiology* 591 (1): 287-302.

Van Soest, P. J, Robertson, J. B. and Lewis, B. A. 1991. Methods of dietary fibre and non-starch polysaccacarides in relation to animal nutrition. *Journal of Dairy science,* 74: 3583-3597

Wodajo, H.D., Gemeda, B.A., Kinati, W., Mulem, A.A., van Eerdewijk, A. and Wieland, B., 2020. Contribution of small ruminants to food security for Ethiopian smallholder farmers. *Small Ruminant Research*, *184*, p.106064.