Bio Efficacy of Benth Leaf (pouzolsia Guinnensis) Extracts Against Gastro Intestinal Related Diseases in Growing Rabbits

By

K.T. AKANNI and O.A. OSINOWO
E-mail: kalerabbit@yahoo.com
Study Area: Animal Breeding & Genetics
Department Agricultural Education,
Federal College of Education, Abeokuta, Nigeria.

Abstract
The study examined the level of the use of Benth Leaf (Pouzolsia guinnensis Benth) leaf extracts against gastro intestinal related diseases in growing rabbits. A total of 35 each of six weeks old averaged 550g-600g body weight of three pure lines; the New Zealand White (NZW); American Chinchilla (CHL) and the FCEABK-?, a newly developed rabbit strains were used for the study. These rabbit lines were sourced from the Tetfund funded Rabbit Breeding and Multiplication research farm, Federal College of Education, Abeokuta, Ogun State. The dosage levels of the Pouzolsia guinnensis (Pg) extracts were: 0ml(control), 5ml, 7.5ml, 10ml and 12.5ml per250ml of drinking water per day for a period of six weeks. Eggs (oocytes) per gram of feacal samples of each rabbit strain were collected and analyzed using modified McMaster Technique. Blood samples were also collected at 2 weeks intervals. There was a significant (P<0.05) general decrease in frequency counts and percentages of gastro intestinal worm oocytes and enhanced haematology and serum traits across the dietary treatments due to reduced GI infestation in the animal body. Recommendations were therefore made that the use of Pouzolsia guinnensis (Pg) leaf extracts at 7.5ml/250ml inclusion that had a concomitant negative effect against GI infections, brought about by reduced GI oocytes and enhanced blood chemistry and should be employed by rabbit farmers and breeders as against gastro intestinal infections in growing rabbits; since it is efficient, cheap, non-toxic and readily available.

Key Words: **Benth Leaf, extracts, gastro intestinal infections, Faecal sample, Rabbit strains.**

Introduction

Due to the awareness of the nutritional importance of rabbit meat, there have been increased demand for, and consumption of rabbit meat especially during the Covid 19 pandemic period when there was food shortage during lockdown crisis with consequent sharp growth of the rabbit industry. Report from FAO (Trocino et al., 2018) however, stated that from year 1998 to 2020 globally, 980,785,000 rabbits were slaughtered in 2016 and 1,428,085 tons of rabbit meat were produced (compared with the global meat production of 329,890,425 tons). Asia is recorded the largest rabbit meat producer in the world (approx. 73% of the global market) followed by Europe, Africa, and the Americas whose share of the global market is approximately 20%, 6.1%, and 1%, respectively (Trocino et al., 2018).

Rabbit as a veritable tool in ameliorating animal protein supply has also been reported inadequate because of its low population in relation to human population, low level of animal productivity in terms of slow growth rate, slow productive capacity and reproductive ability, as influenced by gastro intestinal infection and thus has direct influence on general well-being and health of the ever-increasing population (Adejinmi et al., 2005; Fapohunda et al., 2005).

Rabbit productive potentials is known to be negatively influenced by a number of gastro internal parasites ranging from round worms (nematodes), tapeworms (cestodes) and protozoa (coccidians). A condition which is on the alarming rate across the world. They do not generally cause clinical problems in the adult rabbit but may be associated with poor haircoat, weight loss and a perineal dermatitis. By contrast, young rabbits may be affected by heavy infestations of these worms, particularly at the time of weaning when the microflora of the hindgut is yet to establish itself. Affected young rabbits may show signs of diarrhoea, lethargy, anorexia and weight loss (Glen, 2008). However, economic losses due to helminths infestation in rabbit production occurs in a variety of ways: they cause losses through lowered fertility, reduced work capacity, involuntary culling, a reduction in feed intake, reduced efficiency of feed utilization and lower weight gains, lower milk production, treatment costs, and rearing. A control measure using sensitive and accurate diagnosis techniques based on the reduction in faecal egg counts (FEC) is necessary to control helminth infestation in order to reduce the loss and enhance their performance in terms of productive ability and reproductive capacity (Emiru et al.,2013). Indeed, the use of FEC in micro livestock such as rabbits and other livestock species has several important purposes (Bosco 2014); to determine whether animals are infected and to estimate the severity of infection, to investigate the need for the treatment of helminth infection cases to improve their health with the resulting increase of productive performance: to predict pasture contamination by parasitic eggs and lastly, to determine the efficacy of antihelminths as well as monitoring control programmes.

Prophylaxis is the prevention and treatment of diseases. Reducing risk factors through a healthy lifestyle prevention and maintenance disease conditions through healthy diet, and correcting animals' ill effect by dietary manipulation, using natural plant (herbal) against gastro intestinal infections in rabbits is regarded as an important part of primary prophylaxis. This also includes vaccinations and routine health screenings especially for growing and adult rabbits. Secondary Prophylaxis are measures that are taken to prevent the recurrence of a health problem or internal and external damages that has already occurred, such as reviewing the environmental or those risk factors to prevent re-occurrence of gastro intestinal attack in young rabbits (Musich et al.,2016; C.D.C. 2022).

However, if there is detection in animal body, an underlying gastro intestinal disease related process, the goal is to catch it in its earliest stages, especially when the young rabbits are not feeling ill or having any symptoms. Early diagnosis means it is manageable, treatable, and possibly even curable. Wholistic measures should therefore be taken to prevent a minor issue from becoming a major health problem consequently inhibiting the survivability, productive capacity, reproductive ability and overall performance of potentially growing rabbits (Silverman et al., 2016; Martins et al., 2018).

The current oral administration of forage prophylactic FENBENDAZOLE against gastro intestinal infections in water has not been fully evaluated and recommended. It is currently and mainly used as a treatment for infected individuals. The study also justifies that since animals' ill effect could be best corrected by dietary manipulation, using natural plant (herbal) in deworming helminth infected rabbits. They are readily available, non-toxic and less expensive compared to some conventional antihelminth drugs as noticed with the problems of toxicity, efficacy cost and sometimes not readily available (Morgan et al., 2013; Owusu et al., 2016).

Benth leaf (Pouzolsia guinnensis) alongside other classes of Pouzolsia species are considered as a perennial herb. Their leaves contain flavone, flavonoids, tannin, carotene, carotenoids, ascorbic, tartaric, malic and pectic acids, gum, minerals and -?-D-glucopyranoside; ?-sitosterol3-O-?their salts; quercetin, vitexin, isovitexin, phylanthin, metyl sterate and oleanolic acid (Fu et al., 2012; Nguyen et al., 2019). The leaf powder also contains carbohydrates, gums, reducing sugar, alkaloids, steroids, glycosides, tannins, flavonoids and saponins (Saha and Paul, 2012; Nguyen et al., 2019). Phytochemical derived from plant consisting of phenols and flavonoids possess antioxidant properties, which are useful to scavenge reactive oxygen species (Hossain et al., 2013). Benth leaves are anthelminthic and vulnerary; used as a cicatrizant for gangrenous ulcers, in syphilis and gonorrhea. Poultice of the herb is applied to sores, boils and to relieve stomachache. Steroids (Stigmasterol and ?-Sitosterol) and triterpenoid (friedelin) has antitumor or pesticidal activity

(Shekhar et al., 2018). The plant Pouzolzia guinnensis (Pg) was claimed to be useful in treating snake poison in the Indian system of medicine (Ahmed et al., 2010). Pouzolzia guinnensis plant can be used as fresh or dried plant, decoction drunk to treat cough, pulmonary tuberculosis, sore throat, enteritis, dysentery (Shekhar et al., 2018). Extracts of Pouzolzia guinnensis possessed antibacterial, antifungal and cytotoxic activities (Saha et al., 2012; Saha and Paul, 2012). Pouzolzia guinnensis extracts are very good antibacterial activity against Staphylococcus aureus and Escherichia coli (Saha et al., 2012). Therefore, Pouzolzia guinnensis was used to control fly larvae during food processing due to insecticidal activity (Nguyen et al., 2017). This study therefore, seeks to evaluate the bio-efficacy of Benth leaf (Pouzolzia guinnensis) extracts as prophylaxis against gastro intestinal related diseases in growing rabbits.

Materials and Method

The experimental rabbits comprised 35 each of six weeks old averaged 550g-600g body weight of three pure lines; the New Zealand White (NZW); American Chinchilla (CHL) and the FC EABK-?, a newly TETFund funded developed rabbit line were used for the study. These rabbit lines were sourced from the on-going TETFund funded Rabbit Breeding and Multiplication research farm, Federal College of Education, Abeokuta, Ogun State. The rabbits were diagnosed to determine the frequency counts (FEC) and percentages in reduction of gastro intestinal related diseases infection at the initial stage of the experiment through the examination of the feacal samples collected from the experimental animals. Fresh leaves of Pouzolsia guinnensis (Pg) were cut and thoroughly washed; chopped into smaller pieces and later transferred into juice extractor machine. The leaf extract collected was then diluted with drinking water at varying proportions. The dosage levels of the Pouzolsia guinnensis extract (PGE) according to treatment (T) effects were: T1 = 0ml PGE), T2 = 5ml PGE, T3 = 7.5ml PGE, T4 = 10ml PGE and T5 = 12.5ml PGE in 250ml of drinking water per day throughout the course of the study. Treatment one (0ml/250 ml water) served as a control to all the five (5) dietary treatments.

Faecal samples collection and laboratory processing:

Feacal samples of each rabbit strain were collected using swab stick. The modified McMaster technique (Gearcelin et al., 2021) was employed using three grams of faeces putting into a container adding 42 ml of saturated sodium chloride solution (NaCl, specific gravity?=?1.2) diluted in 1:15 ratio. The faecal suspension was thoroughly homogenized and strained three times through a wire mesh (aperture of 250 m) to remove large debris. The strained suspension was collected in a bowl and thoroughly mixed by pouring it 10 times in one bowl to another. Then, 0.5 ml

aliquots were added to each of the two chambers of a McMaster slide. After 10-12minutes, the different helminth species egg (oocyte) counts were conducted under the two grids (volume?=?0.3 ml) of the McMaster slide (Cringoli et al., 2004) under a light microscope using a 100× magnification. The faecal collection (FEC) values, expressed as Egg Per Gram (EPG) or Oocyte Per Gram (OPG) of helminths, were generated by multiplying the total number of eggs by 50 (McM50). This technique was repeated at two weeks interval, starting from first day of the experiment for a period of 6 weeks.

Performance characteristics:

The rate of frequency counts (FEC) and percentages in reduction of gastro intestinal related diseases infection per strain of rabbits in each of the five dietary treatments was taken and recorded. Blood samples were also collected at four weeks interval (i.e., 0, 2, 4 and 6 weeks) in order to determine the effect of the dietary treatments on the blood chemistry of the infected rabbits. Data collected on oocyte frequency counts (FEC) were arc-transformed into percentages. The strain and treatment effects on blood parameters were estimated from two-way analysis of variance with sub-samples using the General Linear Model of SAS (2019). Significant differences among means were separated. All effects except error terms were considered as fixed effects.

Results

Table 1 presents the Least Square Means ± Standard Error Mean (SEM) of the analysis of variance of the trends in the reduction of the various species of gastro intestinal worms (Cestodes, Nematodes and Coccidians) detected in the feacal samples collected and observed in each of the three strains of rabbits used in the study. Results revealed that there were significant ($P<0.05$) differences in the population of oocytes in each class of worms detected. Four varieties of cestodes detected include Taenia pisifarmis, Taenia lydatigena, Taenia serialis and Taenia multicept; three varieties of Nematodes also recorded include Trichostrongylus reortaeformis, Passalurus ambiguous and Strongylus papiullosus. The only coccidian specie detected in the faecal sample was Emeria steidae. Findings from the Table 1 also showed that within and across the rabbit strains, there was significant ($P<0.05$) differences in the abundance of the cestode oocytes (eggs) with the New Zealand White having the highest value, this was followed by Chinchilla while the FCEABK- ? (K-?) had the least value with the corresponding frequency count (FC) of 11799.37 ± 15.35, 9250.22 ± 0.01 and 5108.02 ± 0.01 respectively.

As noticed in the results of population of Nematodes detected, Chinchilla on the other hand had the highest population of Nematodes with the frequency count of 6677.23 ± 0.01 followed by K-? (4590.14 ± 0.01) the least frequency count value of

4702.36 ± 6.28 was seen in the New Zealand White rabbits. The highest frequency Faecal count (FEC) of coccidian species detected in the study was obtained from the Chinchilla, the next highest score value was noticed in New Zealand White rabbits while the lowest score was observed in K-? in descending order of 123.12 ± 0.01, 114.02 ± 1.21 and 113.01 ± 0.94 respectively. (Table 1). A critical review revealed that there was overall effect of Pouzolsia guinnensis extracts (PGE) in all the dietary treatment which brought about a concomitant reduction in the population of helminth oocytes across the rabbit strains as the rabbits advanced in age from week 2 - week 6.

As presented in table 2, which showed the Least Square Means ± SEM of percentage reduction in helminth population across the rabbit strains. There was generally, high level of percentage reduction in GI oocytes among the three strains of rabbits considered in the study, due to the anti gastro intestinal property of (PGE) used as dietary treatments T2 to T5 respectively. The patterns in the reduction of helminth oocytes as observed in this study favoured the rabbit strains in the dietary treatments two (5.0ml/250ml) and three (7.5ml/250ml) PGE which had the lowest overall frequency counts (FC) percentage reduction of 6.01 ± 0.11% at the 6th week compared to the rabbits in treatment one (0ml/ 250 Pg) which recorded the highest percentage reduction of 79.84 ± 1.10%, 85.02 ± 1.04% and 88.59 ± 1.06% for Cestodes, Nematodes and Coccidians respectively. Similar trends were also observed in both Chinchilla and K-? strains respectively.

The results in Tables 3 and 4 respectively indicated that the highest blood chemistry picture of the rabbit strains used, in terms of higher values of HBC, PCV, RBC, WBC among others. Thus, the use of Pouzolsia guinnensis extract (PGE) at 7.5ml/250ml (water) inclusion could be premised as a standard prophylaxis dose for the prevention and the treatment of gastro intestinal infections in growing rabbits for enhanced performance and productivity.

Table 1: Least Square Means ± SEM of gastro intestinal oocytes reduction as affected by rabbit genotypes.

STRAIN	TYPES OF WORMS DETECTED	Mean FFC (week 0)	Mean FFC (week 2)	Mean FFC (week 4)	Mean FFC (week 6)
NZW	Taenia pisiformis	2233.02± 3.11^{b}	1111.21± 1.34^{a}	674.21± 0.93^{a}	13.12± 0.08^{c}
	Taenia lydatigenia	2657.22± 3.52^{a}	1071.14± 1.20^{a}	701.23± 0.94^{a}	17.00± 0.12^{b}
	Taenia serialis	2456.01± 3.41^{b}	1001.11± 1.20^{b}	778.15± 0.98^{a}	27.01± 0.12^{b}
	Taenia multicept	4453.12± 5.31^{a}	1722.23± 1.49^{a}	900.13± 1.20^{a}	38.02± 0.19^{a}
	CESTODES	**11799.37± 15.35^{a}**			
	Trichostrongylus reortaeformis	1234.02 ± 1.58^{b}	884.02± 1.08^{b}	408.22± 0.37^{b}	14.01± 0.08^{a}
	Passalurus ambiguous	2345.12± 3.42^{a}	1005.12± 1.10^{b}	648.11± 0.88^{b}	24.14± 0.10^{a}
	Strogylus papillosus	1123.22± 1.28^{c}	908.33± 1.08^{b}	438.17± 0.34^{b}	30.03± 0.12^{a}
	NEMATODES	**4702.36± 6.28^{b}**			
	Emeria steidae	114.02± 1.21^{b}	67.23± 0.43^{b}	32.16± 0.52^{c}	22.22± 0.11^{a}
	COCCIDIA	**114.02± 1.21^{b}**			
CHL	Taenia pisiformis	3342.22± 4.21^{a}	793.00± 1.00^{c}	403.01± 0.31^{c}	22.02± 0.12^{a}
	Taenia lydatigenia	1231.12± 1.28^{b}	899.65± 1.05^{b}	501.22± 0.38^{c}	33.10± 0.18^{a}
	Taenia serialis	2543.11± 2.25^{a}	1200.55± 1.33^{a}	724.18± 0.87^{b}	42.03± 0.20^{a}
	Taenia multicept	2134.11± 2.14^{b}	1211.44± 1.30^{b}	601.27± 0.81^{b}	23.03± 0.12^{b}
	CESTODES	**9250.22± 0.01^{b}**			
	Trichostrongylus reortaeformis	2113.15± 2.13^{a}	1207.25± 1.22^{a}	864.35± 0.92^{a}	13.02± 0.08^{b}
	Passalurus ambiguous	2221.21± 2.14^{b}	1211.28± 1.21^{a}	668.27± 0.64^{a}	19.01± 0.09^{b}
	Strogylus papillosus	2343.12± 2.18^{a}	1178.17± 1.19^{a}	701.16± 0.68^{a}	12.00± 0.07^{b}
	NEMATODES	**6677.23± 0.01^{a}**			
	Emeria steidae	123.12± 0.93^{a}	74.54± 0.62^{a}	37.32± 0.29^{a}	9.21± 0.08^{b}
	COCCIDIA	**123.12± 0.01^{a}**			
. ..	Taenia pisiformis	1421.11± 1.56^{c}	901.33± 1.10^{b}	623.44± 0.83^{b}	14.00± 0.08^{b}
	Taenia lydatigenia	1233.02± 1.48^{b}	700.45± 0.90^{c}	593.11± 0.71^{b}	12.03± 0.07^{c}
	Taenia serialis	1211.13± 1.44^{c}	693.31± 0.78^{c}	411.08± 0.46^{c}	9.00± 0.07^{c}
	Taenia multicept	1243.04± 1.42^{c}	888.38± 1.06^{c}	408.35± 0.44^{c}	11.01± 0.08^{c}
	CESTODES	**5108.22± 0.01^{c}**			
	Trichostrongylus reortaeformis	1232.06± 1.46^{b}	769.21± 0.89^{c}	311.26± 0.39^{c}	13.02± 0.06 ^{b}
	Passalurus ambiguous	1213.04± 1.43^{c}	699.33± 0.84^{c}	328.18± 0.39^{c}	17.01± 0.08^{c}
	Strogylus papillosus	2145.04± 2.06^{b}	893.22± 0.95^{c}	334.15± 0.40^{c}	9.01± 0.04^{c}
	NEMATODES	**4590.14± 0.01^{c}**			
	Emeria steidae	113.05± 0.94^{b}	59.36± 0.41^{c}	36.03± 0.29^{b}	2.33± 0.02^{c}
	COCCIDIA	**113.05± 0.94^{b}**			

$^{a,b,c:}$ Means in the same column with different superscripts are significantly different (P<0.05)

FFC = Frequency faecal count
NZW = New Zealand White
CHL = Chinchilla
K-α = FCEAB - α

Table 2: Least Square Means ± SEM of overall percentages of G.I. oocytes reduction in rabbit strains as influenced by dietary treatments

STRAIN	Helminth species	T1 % Red	T2 % Red	T3 % Red	T4 % Red	T5 % Red
NZW	Cestodes	79.84±1.10^{c}	41.95±0.25^{c}	36.25±0.78^{b}	12.87±0.35^{a}	1.12±0.05^{a}
	Nematodes	85.02±1.04^{b}	69.95±0.34^{a}	37.36±0.80^{a}	14.58±0.36^{a}	1.70±0.02^{a}
	Coccidia	88.59±1.06^{b}	66.33±0.33^{b}	31.68±0.74^{c}	21.78±0.39^{a}	5.94±0.06^{b}
CHL	Cestodes	86.08±1.05^{b}	51.52±0.30^{b}	27.99±0.58^{c}	12.99±0.34^{a}	0.97±0.00^{b}
	Nematodes	83.73±1.11^{c}	59.86±0.31^{b}	37.17±0.61^{a}	11.63±0.27^{b}	0.73±0.01^{c}
	Coccidia	95.65±1.16^{a}	71.84±0.51^{a}	45.63±0.72^{a}	17.47±0.38^{b}	8.73±0.03^{a}
. ..	Cestodes	94.65±1.15^{a}	65.12±0.38^{a}	41.64±0.68^{a}	13.54±0.32^{c}	0.94±0.01^{c}
	Nematodes	94.53±1.14^{a}	54.41±0.32^{c}	22.19±0.51^{b}	6.22±0.19^{c}	0.85±0.01^{b}
	Coccidia	87.50±1.12^{c}	59.18±0.30^{c}	36.73±0.59^{b}	6.12±0.17^{c}	2.04±0.02^{c}

$^{a,b,c:}$ Means in the same column with different superscripts are significantly different (P<0.05)

FFC = Frequency faecal count
Red = Percentage reduction
NZW = New Zealand White
CHL = Chinchilla
K-α = FCEAB - α

Table 3: Least Square Means ± SEM of overall blood profile of rabbit strains as influenced by dietary treatments

STRAIN	TRT	N	HBC	PCV	RBC	WBC	MCV	MCH	GLU
NZW	T1	32	11.30±0.03^{b}	40.90±0.00^{b}	4.30±0.42^{c}	4.05±0.35^{c}	54.55±5.35^{c}	22.65±2.15^{b}	60.30±1.17^{b}
	T2	35	13.14±0.10^{a}	41.55±0.05^{b}	6.94±0.44^{a}	7.39±0.23^{ab}	66.20±0.80^{a}	22.35±3.05^{b}	60.60±0.09^{b}
	T3	32	16.89±0.00^{a}	51.96±0.6^{a}	7.07±0.03^{a}	8.53±0.33^{a}	72.70±2.60^{a}	34.15±0.05^{a}	87.10±0.00^{a}
	T4	33	13.14±0.10^{a}	41.55±0.05^{b}	6.94±0.44^{a}	7.39±0.23^{ab}	66.20±0.80^{a}	22.35±3.05^{b}	60.60±0.09^{b}
	T5	30	11.69±0.15^{b}	41.45±1.09^{b}	4.62±0.12^{c}	4.45±0.15^{bc}	58.62±0.02^{c}	20.33±0.03^{c}	46.30±1.20^{c}
CHL	T1	35	13.10±0.18^{bc}	39.16±6.13^{bc}	5.95±0.45^{ab}	6.15±1.65^{b}	65.65±1.35^{b}	32.70±7.10^{a}	53.05±3.05^{bc}
	T2	34	14.81±0.15^{b}	41.40±0.10^{b}	7.61±0.01^{a}	7.00±2.00^{ab}	67.43±0.02^{b}	33.30±0.10^{a}	70.00±0.00^{b}
	T3	33	16.20±0.15^{a}	51.60±1.10^{a}	7.90±0.05^{a}	8.15±1.65^{ab}	81.55±5.35^{a}	33.70±2.15^{a}	80.00±1.07^{a}
	T4	31	14.81±0.15^{b}	41.40±0.10^{b}	7.61±0.01^{a}	7.00±2.00^{ab}	67.43±0.02^{b}	33.30±0.10^{a}	70.00±0.00^{b}
	T5	29	11.18±0.18^{c}	33.46±0.16^{c}	4.02±0.17^{c}	5.92±0.00^{c}	67.75±0.85^{c}	28.50±4.10^{ab}	48.36±1.21^{c}
. ..	T1	35	12.40±0.11^{c}	44.95±1.09^{b}	4.64±0.11^{b}	5.45±0.15^{bc}	66.00±0.01^{b}	23.05±3.05^{b}	51.00±5.00^{bc}
	T2	35	14.25±0.10^{b}	51.40±0.01^{a}	7.28±0.00^{a}	8.63±0.30^{a}	77.05±0.15^{a}	28.50±4.10^{ab}	68.36±1.31^{b}
	T3	35	16.64±0.01^{a}	52.35±1.00^{a}	07.74±0.04^{a}	8.67±0.00^{a}	80.65±0.00^{a}	34.70±0.10^{a}	73.00±0.00^{a}
	T4	30	14.25±0.10^{b}	51.40±0.01^{a}	7.28±0.00^{a}	8.63±0.30^{a}	77.05±0.15^{a}	28.50±4.10^{ab}	68.36±1.31^{b}
	T5	28	11.82±0.12^{c}	41.62±0.09^{c}	5.06±0.01^{b}	4.95±0.05^{c}	66.01±3.10^{a}	28.66±2.14^{ab}	45.70±0.50^{c}

$^{a,b,c:}$ Means in the same column with different superscripts are significantly different (P<0.05)

HBC = Haemoglobin Concentration (gdl^{-1}); PCV = Packed Cell Volume (%)
RBC = Red Blood Cell ($x10^6mm^{-3}$); WBC = White Blood Cells ($x10^6mm^{-3}$)
MCV = Mean Cell Volume (Fl); MCH = Mean Cell Haemoglobin (Pg)
GLU = Glucose ($mg^{-}100ml$)
GENOT = Genotype
TRT = Dietary treatment
NZW = New Zealand White
CHL = Chinchilla
K-α = FCEAB – α

Table 4: Least squares means ± SEM of serum chemistry traits of rabbit strains as influenced by dietary treatments

STRAIN	TRT	N	SALBM	SGLBLN	S UREA	STPRT	CHOLST	Na^+
NZW	T1	32	59.20 ± 1.10^{bc}	26.85 ± 0.25^{bc}	31.50 ± 1.20^{a}	60.20 ± 5.90^{ab}	115.65 ± 6.25^{b}	136.00 ± 3.50^{ab}
	T2	35	55.78 ± 0.12^{bc}	33.00 ± 0.25^{b}	24.82 ± 1.20^{b}	56.90 ± 2.80^{b}	90.70 ± 4.20^{c}	111.80 ± 11.80^{bc}
	T3	32	71.10 ± 1.00^{a}	43.77 ± 0.25^{a}	19.20 ± 0.20^{c}	68.90 ± 0.00^{a}	87.90 ± 0.10^{c}	94.75 ± 1.75^{c}
	T4	33	71.10 ± 1.00^{a}	43.77 ± 0.25^{a}	19.20 ± 0.20^{c}	68.90 ± 0.00^{a}	87.90 ± 0.10^{c}	94.75 ± 1.75^{c}
	T5	30	46.00 ± 0.10^{c}	37.20 ± 0.10^{ab}	31.15 ± 1.10^{a}	57.42 ± 0.10^{b}	87.70 ± 0.20^{c}	96.80 ± 7.20^{c}
CHL	T1	35	49.55 ± 1.15^{c}	27.45 ± 0.25^{b}	31.07 ± 1.25^{a}	57.20 ± 5.00^{b}	144.20 ± 0.10^{a}	113.55 ± 15.35^{bc}
	T2	34	66.86 ± 1.19^{ab}	22.88 ± 0.24^{c}	19.05 ± 1.05^{c}	63.75 ± 4.75^{ab}	90.71 ± 5.19^{c}	101.40 ± 8.20^{bc}
	T3	33	55.25 ± 1.15^{bc}	24.26 ± 0.00^{bc}	22.05 ± 0.25^{bc}	59.70 ± 1.00^{b}	107.70 ± 1.10^{b}	136.72 ± 3.27^{a}
	T4	31	49.20 ± 1.10^{c}	27.05 ± 0.00^{b}	31.76 ± 0.00^{a}	57.10 ± 2.90^{b}	114.8 ± 5.20^{b}	132.35 ± 2.85^{ab}
	T5	29	49.78 ± 0.10^{c}	23.12 ± 0.25^{c}	25.02 ± 1.2^{b}	58.65 ± 1.85^{b}	93.95 ± 09.25^{c}	101.25 ± 0.75^{bc}
K-α	T1	35	56.45 ± 1.10^{bc}	27.05 ± 0.25^{b}	28.30 ± 1.10^{ab}	60.75 ± 1.25^{ab}	115.50 ± 8.20^{b}	136.00 ± 0.00^{a}
	T2	35	46.03 ± 1.17^{c}	40.27 ± 0.05^{a}	29.00 ± 0.01^{b}	51.75 ± 8.75^{c}	121.10 ± 01.20^{b}	136.00 ± 0.50^{a}
	T3	35	65.91 ± 0.09^{ab}	23.08 ± 0.28^{c}	19.68 ± 1.25^{c}	63.36 ± 1.57^{ab}	94.24 ± 5.25^{c}	85.15 ± 4.00^{bc}
	T4	30	75.91 ± 0.00^{b}	24.08 ± 0.24^{c}	20.78 ± 1.00^{c}	68.30 ± 1.07^{ab}	99.25 ± 4.25^{c}	115.15 ± 8.05^{bc}
	T5	28	70.55 ± 1.15^{a}	28.45 ± 0.00^{b}	19.03 ± 1.05^{c}	68.55 ± 2.85^{a}	99.85 ± 10.05^{c}	102.80 ± 6.00^{bc}

$^{a,b,c:}$ Means in the same column with different superscripts are significantly different ($P<0.05$)

SALBM = Serum Albumin (gdl^{-1}); SGLBLN = Serum Globulin (gdl^{-1})
S UREA = Serum Urea (gdl^{-1}); STPRT = Serum Total Protein (gdl^{-1})
CHOLST = Cholesterol ($mgdl^{-1}$); Na+ = Sodium ($mgdl^{-1}$)
TRT = Dietary Treatment

Discussion

Significant ($P<0.05$) means ± SEM of the variations in the reduction of gastro intestinal (GI) oocytes among the rabbit strains studied in Tables 1 and 2 is expected. This is due to the differences in the level of tolerance and resistance to gastro intestinal parasites infestation which in turn a function of strain variability. Different strains of animals have differences in the level of tolerance, resistance stability to the varying population of internal parasites in their body (Akanni et al., 2018). Although, some gastro intestinal parasites (cestodes, nematodes and coccidians) are found to be specific and particularly associated with certain species of livestock, even within strain. Glen (2008) working on internal parasites of rabbits had earlier concluded that rabbits are known to be parasitized by a number of different internal parasites. These can be divided into roundworms (nematodes),

tapeworms (cestodes) and protozoa. The author noted further that, the rabbits act as the intermediate host for a number of tape worms, otherwise known as cestodes. In addition to the fact that there are 16 known species of Eimerial parasites affecting rabbits, they rarely pose a problem. Among them, Eimerial steidae is considered as the rabbit specific helminth (protozoan) parasite that causes hepatic (liver) coccidiosis whereas other species of Eimeria infect the rabbit's intestinal tract. Generally, young rabbits are usually most severely affected as they are unlikely to have built up any immunity to the disease. Clinical signs of helminth infection are however reported to be varied among the rabbit strains. This to a large extent, is a function of the severity of the infection and the immune status of the individual rabbits. (Bosch, 2004; Glen, 2008; Randall, 2017; Nguyen et al., 2019). This could have been responsible for the variation in the oocytes frequency count per gram (OPG) of the different species of helminths in the faecal samples collected within and across the rabbit strains.

Genetic and environmental reasons may have accounted for the variability in the blood chemistry traits at different ages (weeks) among the rabbit strains considered during the study. There are wide variations in the trends. The considerable performance in the blood chemistry patterns obtained in this study implied that reduction in Gastro-Intestinal infections in animal body tissues has a direct and positive influences on the performance characteristics and economic values of farm animals especially in rabbits and that rabbits should be wormed on regular basis for optimal productivity and general well-being of the human populace. It is basically for this reason, in order to minimize health risks to humans, that the commercial and breeder rabbits should be wormed every 6 weeks of age. Currently, there is no economic justification to treatment for the commercial rabbits against GI infestation especially using prophylaxes such as fenbendazole (worm expeller) and some conventional antihelminth drugs as noticed with the problems of toxicity, efficacy, cost and sometimes not readily available. Hence, the strategy towards dietary manipulations using locally available herbs such as Pouzolsia guinnensis (Benth) leaf extract becomes imperative. (Randall, 2017). They are readily available, nontoxic and inexpensive.

However, the results obtained in the haematology and serum traits among the rabbit strains also followed a continuous reduction in frequency in counts of oocytes per gram (OPG) of GI in the faecal samples in treatments 2 and 3 had brought about a concomitant better blood picture, as shown in Tables 3 and 4 respectively. This could have equally brought to a conclusion that at threshold reduction in helminths population, there is better performance in terms of feed intake and efficiency of feed utilization with concomitant better blood picture of the animal.

Hematological system in rabbits is highly susceptible to drug-induced toxicity due to its vital position and functions. (Woode et al., 2011). The reduction in

haematology (HBC, PCV, RBC, WBC, MCV, MCH etc.) from 4th to 6th week of the dietary treatments in all the extract-treated groups may be due to the direct destructive effect of the extract on these cells or their impaired production in the hematopoietic tissues (bone marrow). Moreover, the reduction of WBC following extract treatment might be because of the mobilization of the leukocytes to the tissues surrounding the blood (Akah et al., 1998; Woode et al., 2011).

However, higher values obtained in blood serum traits such as (S ALBM, S GLBLN, S UREA, CHOLST, Na+ etc. as observed in the dietary treatments four (10ml/250ml) and five (12.5ml/250ml) implied that Pouzolsia guinnensis (Pg) leaf extract may not be safe at higher doses, especially for the management of chronic heart and hepatic related disease conditions like gastro-enteritis and coccidiosis hypertension as could be observed in the frequency counts and percentage reductions in the GI oocytes detected during the frequency and percentage feacal counts in the study.

The practical significance of this is that, for optimal performance and productivity in rabbit industry, accurate diagnosis of gastro intestinal infestation and immune system status of commercial and breeder rabbits are of pivotal importance for both livestock management and the general wellbeing of human population. This includes drug efficacy trial and surveillance of GI related diseases control and elimination programmes as opined by Cringoli et al. (2010). This could have informed the result obtained in this study.

Conclusion

Administration of Pouzolsia guinnensis (Pg) leaf extract at 7.5ml/250m per day level in rabbits' diet was effective in reducing negative effect of the gastro intestinal oocytes. There was much improvement in haematology.

There was a general percentage reduction of helminth oocytes across the treatment from T2 to T3 dosage of Benth leaf extract resulting in a 99.98% reduction of helminths' oocytes while T1 (0g) control had the least percentage oocyte reduction.

Significant ($P < 0.05$) better performance in haematology was noticed and recorded in T3 that had the lowest percentage of GI oocytes reduction. Blood serum traits also followed the similar pattern as obtained in haematology. Thus, the result obtained showed that blood chemistry was due to reduced gastro intestinal oocytes infestation in the growing rabbits studied.

Recommendations

Based on these findings it is therefore recommended that administration of up to 7.5ml/250m (water) Pouzolsia guinnensis (Pg) leaf extract dose per day against gastro intestinal (GI) infections in rabbits' diet is hereby proposed. This is necessary for optimal performance in terms of productive ability and reproductive capacity.

The use of Pouzolsia guinnensis (Benth) leaf extracts at 7.5ml/250m inclusion had a concomitant effect against GI infections as brought about by enhanced blood profile in growing rabbits and should be employed by rabbit farmers as prophylaxis treatment in growing rabbits for enhanced performance and productivity. Since it is cheap, non-toxic and readily available rather than commercial prophylaxis (fenbendazole) that are costly and could sometimes bring about residual effect.

References

Adejinmi, O.O., Fapohunda, J.B, Alonge, G.O, Owosibo, A.O, and Ogunleke, F.O. 2005. Performance of growing rabbits fed Tridax leaf meal (Tridax procumbens) with or without soybean meal in the diets. In: Proceedings of 10th Annual Conf, Animal. Sci. Ass. of Nigeria. (ASAN), Sept.12-15 2005, at University of Edo- Ekiti, Nigeria. pp. 292-294.

Adjahossou, B.S., Naesse, A.V. and Pascal, G. 2017. Importance of Home Gardens in Rural Zone of the Municipality of Abomey-Calavi in south of Republic of Benin. S.A.R. 6(4):150-160.

Ahmed, A., Rajendaran, K., Jaiswal, D., Singh, H.P., Mishra, A., Chandra, D., Yadav, I.K., Jain, D.A. (2010). Anti-snake venom activity of different extract of Pouzolzia indica against Russel viper venom. International Journal of Chem. Tech. Research. 2(1): 744- 751. 3.

Akah, P.A., Orisakwe, O.E., Gamaniel, K.S. and Shittu, A. 1998. Evaluation of Nigerian Traditional medicines on peptic ulcer. J. Ethnopharmacol, 62 (1998), pp. 123-127.ArticleDownload PDFView Record in ScopusGoogle Scholar.

Akanni, K. T., Alabi, A. O., Ogbonna, C. C. and Olufunmilayo Adebambo. 2018a. Productive potentials in pure and crossbred rats in south western Nigeria in Proceedings of 43rdAnnual Conference of Nigeria Society for Animal Production (NSAP) 18 - 22nd March 2018 at New SAAT Lecture Theatre, Federal University of Technology Owerri, Imo State, Nigeria pp. 1071-1074.

Akanni, K.T. 2009. The rabbit as a potential of meat and breeding stock in Nigeria: Training manual for young farmers. A paper delivered at a 6-week summer course on OGEGEP regular training programme and conditional grants scheme organized by the Bureau of Employment Generation Programme (OGEGEP), Ogun State. Theme: Changing lives for good. Held between Monday 7th December - to Friday 5th March, 2010, at Eweje farms Institute, Odeda, Abeokuta, Ogun State.

Akanni, K.T. 2012.Genetic characterization of indigenous and exotic rabbits and their.crosses. (A Ph. D Thesis, Federal University of agriculture, Abeokuta. Pp. 1-320).

Akanni, K.T., S.O. Peters, M. Whetto, A.M. Bamgbose, C.O.N. Ikeobi and Olufunmilayo Adebambo (2012). Sire strain influence on the reproductive performance of three strains of rabbits in south western Nigeria, in Proceedings of 37th Annual Conf. of NSAP 18th - 21st March, 2012 at University of Agriculture, Makurdi, Benue State, Nigeria. pp. 8-10.

Alvarado-Villalobos, M.A. Cringoli, g., and Maureli, M.P. 2017. Flotation techniques (FLOTAC and mini-FLOTAC) for detecting gastrointestinal parasites in howler monkeys. Parasite Vector. 10:1-8.

Barda, B.D., Rinaldi, L. and Lanniello, D. 2013a. Mini-FLOTAC. An innovative direct diagnostic technique for intestinal parasitic infections: experience from the field. PLoS Negl Trop Dis. 7(8):1-7

Bosch, C.H. 2004. Pouzolzia guineensis Benth. [Internet] Record from PROTA4U. Grubben, G.J.H. & Denton, O.A. (Editors). PROTA (Plant Resources of Tropical Africa), Wageningen, Netherlands. http://www.prota4u.org/search.asp. [Accessed 12 Sep 2018].

Bosch, C.H. 2004. Pouzolzia guineensis Benth. [Internet] Record from PROTA4U. Grubben, G.J.H. & Denton, O.A. (Editors). PROTA (Plant Resources of Tropical Africa), Wageningen, Netherlands. http://www.prota4u.org/search.asp. [Accessed 12 Sep 2018].

Bosco A. 2014. The coprological diagnosis of gastrointestinal nematode infections in small ruminants. Doctorate thesis, University Federico II of Naples, p. 144.

C. D. C. 2022. Centers for Disease Control and Prevention; Pre-exposure prophylaxis (PrEP). National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Healthcare Quality Promotion (DHQP).

Cooke BD. 2002. Rabbit haemorrhagic disease: Field epidemiology and management of wild rabbit populations. Rev Sci Off Int Epizoot 21:347-358

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G. and Scala, A. 2004. The influence of flotation solution. sample dilution and the choice of McMaster area (volume) on the reliability of the McMaster method in estimating the fecal egg counts of gastrointestinal strongyles and Dicrocoelium dendriticum in sheep. Vet Parasitol. 123:121-131.

Cringoli, G., Rinaldi, L., Maurelli, M.P.and Utzinger,J. 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic

diagnosis of parasites in animals and humans. Nat Protoc. 5:503-515.DOI:https://doi.org/10.1038/nprot.2009.235. PMID: 20203667.

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, and Scala, A. 2010. The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and Dicrocoelium dendriticum in sheep.

Delibes-Mateos M, Ferreras P, Villafuerte R. 2009. European rabbit population trends and associated factors: A review of the situation in the Iberian Peninsula. Mamm Rev 39:124-140.

Emiru B., Amede, Y. Tigre, W. and Fevera, T. 2013. Epidemiology of gastrointestinal parasites of small ruminants in Gechi District, Southwest Ethiopia. Adv Biomed Res. 7:169-174.

Fapounda, J.B., Adejinmi, O.O. and Ogunleke, F.O. 2005. Performance characteristics of weaner rabbits fed varying levels of cassava leaf meal. Proc. of 10th Annual Conf., Anim. Sci. Ass.of Nig. (ASAN), Sept. 12-15, 2005, Univ. of Ado-Ekiti, Nigeria. Pp. 295-297.

Frunza, G.; Simeanu, D.; Mircea Pop, I.; Boisteanu, P.C.; Stefan, M. 2019. Contributions

Fu, M., Niu, Y.Y., Yu, J., Kong Q.T., (2012). Study on the chemical constituents in Pouzolzia zeylanica. Zhong Yao Cai 35(11): 1778-17781. 12. P.

Gearcelin, G.A., Aadm, D.A., Guenole, C.A., Arsene, C.B., Frejus, T.Z.,and Gilles-Christ, A.A. 2021. A comparison of Mini-FLOTAC and McMaster techniques in detecting gastrointestinal parasites in West Africa Dwarf sheep and goats and crossbreed rabbits, Journal of Applied Animal Research, 49:1, 30-38, DOI: 10.1080/09712119.2021.1876703.

Glen, C. 2008. Discussion on the common endoparasites found in rabbits and how to treat them. (Short communication). https:/www.vettimes.co.uk.

Hossain, M.A., Raqmi, K.A.S., Mijizy, Z.H., Weli, A.M., Riyami, Q. (2013). Study of total phenol, flavonoids contents and phytochemical sreening of various leaves crude extracts of locally grown Thymus vularis. Asian Pacific Journal of Tropical Biomedicine 3 (9): 705-710. 17.

Mandal, S., Patra, A., Samanta, A., Roy, S., Mandal, A., Mahapatra, T.D., Pradhan, S., Das, K., Nandi, D.K. (2013). Analysis of phytochemical profile of Terminalia arjuna bark extract with antioxidative and antimicrobial properties. Asian Pacific Journal of Tropical Biomedicine 3 (12): 960-966.

Martins, C., Godycki-Cwirko, M., Heleno, B., and Brodersen, J. 2018. Quaternary prevention: reviewing the concept. Eur. J. Gen Pract. 2018;24(1):106-111. doi:10.1080/13814788.2017.1422177

Morgan, E.R., Charlier, J., and Biggeri, A. 2013. Global change and helminth infections in grazing ruminants in Europe: impacts. trends and sustainable solutions. Agriculture. 3:484-502.

Morgan, E.R., Chalier, J., Hendrickx G. and Biggeri, A. 2013. Global change and helminth infections in grazing ruminants in Europe: impacts. trends and sustainable solutions. Agriculture. 3:484-502.

Musich, S., Wang, S., Hawkins, K., and Klemes, A. 2015. The impact of personalized preventive care on health care quality, utilization, and expenditures. Popul. Health Manag. 2016;19 (6):389-397. doi:10.1089/pop.2015.0171.

Nguyen Duy Tan and Nguyen Minh Thuy (2018). Optimization of maltodextrin and carrageenan gum concentration added in spray drying process of Pouzolzia zeylanica extract by response surface methodology. Journal of Agriculture and Development 17(3): 78-86.

Nguyen Duy Tan, Le Quoc Viet, Vo Tan Thanh, Nguyen Minh Thuy (2017). Optimization of polyphenol, flavonoid and tannin extraction conditions from Pouzolzia zeylanica L. Benn using response surface methodology. Can Tho University Journal of Science 5: 122-131.

Nguyen Ngoc Bao Chau, Ly Thi Minh Hien, Dang Thi Tinh, Nguyen Bao Quoc (2017). Bioefficacy of leaf extracts from Pouzolzia zeylanica (l.) Benn against diamondback moth Plutella xylostella in Viet Nam. Journal of Science Ho Chi Minh City Open University 7(2): 44-50.

Nguyen, P. M., Van, T.P., Lam, V. H., Ngo, T.T. and Huynh, V. T. 2019. Effectiveness of Pouzolzia zeylanica, Curcuma longa, Piper nigrum, Capsicum annum to Stability of Dried Salted Tilapia during Storage. J. Pharm. Sci. & Res. Vol. 11(4), 2019, 1469-1473.

North, M.K.; Dalle Zotte, A.; Ho_man, L.C. 2018. The effects of quercetin on the Sensorial, Physico-Chemical and Nutritional Characterization of Meat in Flemish Giant Rabbit Breed. Rev. Chim. 2019, 70, 512-517.

Owusu M, Sekyere JO, Adzitey F. 2016. Prevalence and burden of gastrointestinal parasites of Djallonke sheep in Ayeduase, Kumasi, Ghana. Vet World. 9(4):361-364.

Randall, R.P. 2017. A Global Compendium of Weeds. 3rd Edition. Perth, Western Australia. R.P. Randall "Pouzolzia zeylanica ?Weed of: Bananas, Cereals, Orchards & Plantations".

Rouco C, Starkloff A, Delibes-Mateos M, Schubert M, Ro¨del HG. 2012. Differentiation of animals from different age classes by means of pellet size: Assessment of a field method in European rabbits. Mamm. Biol 77:451-45.

Saha, D., Paul, S. (2012). Antifungal activity of ethanol extract of Pouzolzia Zeylanica (L.) Benn. International Journal of Pharmacy Teaching and Practices 3(2): 272-274.

Saha, D., Paul, S., Chowdhury, S. (2012). Antibacterial activity of ethanol extract of Pouzolzia zeylanica (L.) Benn. International Journal of Pharmaceutical Innovations 2(1): 1-5.

SAS, (2019). SAS Users Guide Statical Analysis. Inst. Inc. Cary, North Carolina. Supplementation on New Zealand White Grower Rabbit Carcass and Meat Quality-A short communication. Meat Sci. 2018, 145, 363-366.

Shekhar, K. S., Radha, S. and Kusum S. (2018). Review on medicinal and phytochemical properties of different extract of Turmeric's rhizome. Journal of Pharmacognosy and Phytochemistry 7(3): 874-878.

Silverman, M.G., Ference, B.A, Im, K. 2016. Association between lowering LDL-C and cardiovascular risk reduction among different therapeutic interventions: a systematic review and meta-analysis. JAMA. 2016;316(12):1289-1297.

Trocino, A.; Cotozzolo, E.; Zomeño, C.; Petracci, M.; Xiccato, G.; Castellini, C. 2018. Rabbit production and science: The world and Italian scenarios from 1998 to 2018. Ital. J. Anim. Sci. 2019, 18, 1361-1371.

Woode, E., Poku, R.A., and Abotsi, W.K. 2011. Anxiolytic-like effects of leaf extract of Ficus axasperata Vahl (Moraceae) in mice. West Afr. J. Pharm, 22 (2011), pp. 75-8.View Record in Scopus Google Scholar.