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Dietary Supplementation of Rauvolfia Vomitoria Root Extract as a Phytogenic Feed Additive in Growing Rabbit Diets: Growth Perfomance and Caecal Microbial Population

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Abstract The objective of this study is to evaluate the dietary supplementation of Rauvolfia vomitoria root extract as a phytogenic feed additive in growing rabbit's diets: growth performance and caecal microbial population. A total of thirty (30) weaned rabbits were divided into five treatments with three replicates per treatment of 2 rabbits per replicate in a completely randomized design. Animals in treatment 1 (control) were fed diet basal diet with no Rauvolfia vomitoria root extract (RVME) while T2, T3, T4 and T5 were fed RVME at 20 ml, 40 ml, 60 ml and 80 ml/liter. The experiment lasted for 12 weeks and other management practices were strictly observed. Data collected were used to examine the growth performance (average daily weight gain, average daily feed intake and feed conversion ratio) and caecal microbial population (Escherichia coli and lactobacilli count). Average daily weight (ADWG) were significantly ($P < 0.05$) different among the treatments. Feeding rabbits RVME from 20 ml to 80 ml/liter tends to increase the average daily feed intake (ADFI) though not a significant rate ($P > 0.05$). Highest mortality was recorded in T1 with (1.10 %), none was recorded in the other treatments ($P < 0.05$). Lactobacilli and Escherichia coli count were influenced by the dietary treatments ($P < 0.05$). Escherichia coli count significantly reduced as the level of RVME increases while increasing RVME from 20 ml to 80 ml/liter tend to increase lactobacilli count ($P < 0.05$). It was concluded that RVME contains several bioactive chemicals and could be fed to growing rabbits at 80 ml/liter without any deleterious effect on the performance and caeca microbial population of the animals.

Keywords: Growing rabbits, Rauvolfia vomitoria root, performance, phytochemicals.

INTRODUCTION

Phytogenics are natural plant based substances used effectively in animal health and nutrition to improve livestock's health, growth and performance (Sripathy, 2019). They are also regarded as natural growth promoters (NGPs) as an alternative to antibiotic growth promoter (AGPs) because of the emergence of drug resistance microorganisms, side effects of antimicrobials and the harmful residual toxicity effects of drugs observed in the food chain (Okitoi et al., 2007; Adu et al., 2009). Studies have also shown that the growth promoting effect of antibiotics was correlated with the decreased activity of bile salt hydrolase, an intestinal bacteria produced enzyme that exerts negative impact on the host fat digestion and utilization (Lin, 2014). Scientific reports have shown that plants exhibit wide range of biological activities such as: anti-inflammatory, antioxidants, anti-bacterial, antifungal, hepatoprotective, cytotoxic, immunostimulatory, miracicidal, antiviral and cardiovascular properties (Paul et al., 2011; Amole et al., 1998; Khan et al., 2002; Stephen and Joseph, 2011; Satheesh et al., 2012 and Owolabi et al., 2007). According to WHO (1998), there are over 250, 000 species of medicinal plants globally, some are yet to be explored. Among the potential herbal plant is Rauvolfia vomitoria.

Rauvolfia vomitoria an Apocynaceae, is a medicinal plant widely distributed all over the world especially in Asia and West African countries (Olajumoke et al., 2012). It is a tree that grows to a height of about 15 meters and is found in most low land forest. Traditionally, the plants (leaves root and stem bark) are used for the treatment of malaria, rheumatism, hypertension, skin disease, stomach disorders and mental disorder (Ibironke and Olusola, 2013). The plant contain several bioactive chemicals like alkaloids,

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flavonoids, glycosides, saponins, phenols and reducing sugars (Satheesh et al., 2012; Akinyeye et al., 2010; Ajayi et al., 2010). Root and stem bark are commonly known for their aphrodisiac, antisporic, abortive and insecticidal properties also for the antihelminthic, apercent, dysenteric, astringent, cardio tonic, diaphoretic, hypotensive, vulnerary and febrifugic potential (Olajumoke et al., 2012).

Rauvolfia vomitoria has numerous pharmacological properties and could be considered as an alternative to antibiotics, this will further encourage food safety and increase productivity in animals. Therefore, this experiment was carried out to evaluate the dietary supplementation of Rauvolfia vomitoria root extract as a phytogenic feed additive in growing rabbit's diets: growth performance and caecal microbial population.

MATERIALS AND METHODS

STUDY AREA

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Institute, Gujarat, India during the month of April to June, 2020.

COLLECTION, IDENTIFICATION, PREPARATION AND ANALYSIS OF RAUVOLFIA VOMITORIA ROOT EXTRACT

Fresh roots from mature Rauvolfia vomitoria tree were harvested from different plants within Sumitra Research Institute Gujarat, India and authenticated by a certified crop taxonomist (Dr. Maureen Sharma). The roots thoroughly washed with running tap water to remove dirt and later cut into bits and allowed to dry under shade for 10 days to retain the bioactive chemicals in the sample. The dried samples was blended into fine powder using mortar and pestle and stored in a well labeled air tight container for further analysis. Rauvolfia vomitoria extract was prepared by soaking 200 g of the sample in 1000 liters of water in the refrigerator at 4oC for 48 hours. The mixture was filtered using WhatMan No 1 filter paper to obtain the filtrate Rauvolfia vomitoria root extract (RVME).

Moisture, crude protein, crude fibre, ash, ether extract and carbohydrate of the samples were determined using methods of the Official Analytical Chemist (AOAC, 2000). Phytochemical evaluation of tannins, alkaloids, saponins, flavonoids, phenols, oxalate, glycosides, steroids and terpenoids were estimated using methods described by Harbone (1973), Odebiyi and Sofowora (1978), Boham and Kocipai (1974). Vitamin analysis was carried out according to the methods outlined by Ngozi et al. (2017).

ANIMAL MANAGEMENT AND FEED FORMULATION

Thirty (30) weaned rabbits of mixed breed and sex between 6-7 weeks with an average weight of 530.9 and 533.0 grams were used for the experiment; they were sourced from an open market in India. The animals were housed in an all wired cage measuring (15 × 12 × 25 cm) and equipped with feeders and drinkers. Prior to the commencement of the experiment, pen and cages were properly disinfected and all other biosecurity measures were strictly observed. They were divided into five treatments with three replicates per treatment of 2 rabbits per replicate in a completely randomized design. Rabbits were allowed two weeks adjustment period during which they were fed with basal diet (morning and evening) and given prophylactic treatment with Oxytetracycline administered intramuscularly and Ivermectin given subcutaneously adhering strictly to the package insert. Animals were fed twice daily between 7:30 am and 3:30 pm. Fresh feed and water were provided ad libitum and all other management practices were strictly observed throughout the experiment which lasted for 84 days.

The basal diet was formulated to meet the nutrient requirements of growing rabbits according to NRC (1977).

Treatment 1: Basal diet + 0 % RVME
Treatment 2: Basal diet + 20 ml/litre RVME
Treatment 3: Basal diet + 40 ml/litre RVME
Treatment 4: Basal diet + 60 ml/litre RVME

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Treatment 5: Basal diet + 80 ml/litre RVME

MEASUREMENTS

Feed intake (g) was determined by subtracting feed left over from feed served, it was estimated for each of the replicate daily.

Weight gain (g) was calculated by finding the difference between initial weight and final weight at the end of the experiment.

Average daily weight gain (ADWG) = Final body weight – Initial body weight / Total days of the experiment.

Average total feed intake (ADFI) = Feed intake / Total days of the experiment.

Feed conversion ratio (FCR) = feed intake (g)/weight gain (g).

CAECA MICROBIAL POPULATION

At the end of the experiment total caecal bacterial population was determined according to the method outlined by American Public Health Association (APHA, 1960). Isolation of E. coli and lactobacilli bacteria was carried out by method described by El-Ghalid et al. (2019).

STATISTICAL ANALYSIS

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (18.0) and significant means were separated using Duncan multiple range tests (Duncan, 1955). Significant was declared if $P \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 reveals the percentage composition of experimental diet. The feed contained crude protein (17.08 %), crude fibre (9.87 %), ether extract (3.52 %), calcium (0.61 %), phosphorus (0.33 %) and energy (2500.1 kcal/kg). This conforms to the findings of Safwat et al. (2015); Abubakar et al. (2015). The crude protein and crude fibre is with the recommended range by Aduku and Olukosi (1990). Ether extract fall within the recommended ranges by Abu et al. (2016).

The energy value is in close agreement with the findings of Unigwe et al. (2016) who examined the effect of neem leaf (Azadirachta indica) meal on the growth performance of rabbits. However, the proximate values of the experimental diets were within the nutritional requirement of rabbits according to NRC (1977); Fielding (1991). Inadequate energy, protein or micronutrients in the diet may impair performance of rabbits (Niyi, 1997). A balanced diet containing prerequisites amounts of energy, proteins, fat, minerals, vitamins and water are essential for rabbits reared under intensive production (Aduku and Olukosi, 1990).

Adequate fibre in the diet of animals lowers serum cholesterol level, aids digestion and prevents the risk of coronary heart disease (Ishida et al., 2000; Alagbe et al., 2000). Adequate intake of dietary ether extracts or fats provides energy, improves palatability and transport of fat soluble vitamins in the body (Aiyesanmi and Oguntokun, 1996; Shittu and Alagbe, 2020; Musa et al., 2020). Proteins play a central role in cell growth and strengthening the immune system (Alagbe, 2019; Omokore et al., 2019; Oluwafemi et al., 2020). Energy is required by rabbits for the contraction of muscles which enable the rabbit to move, build up their physiology and make products such as fur, milk and meat etc (Fielding 1991).

Ingredients	Quantity (Kg)
Maize	29.40
Wheat offal	55.00
Palm kernel meal	4.25

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Soya bean meal	10.00
Bone meal	0.40
Limestone	0.20
Lysine	0.10
Methionine	0.10
***Growers Premix**	0.25
Salt	0.30
Total	100.00

CALCULATED ANALYSIS (%)

Crude protein	17.08
Crude fibre	9.87
Ether extract	3.52
Calcium	0.61
Phosphorus	0.33
Ca :P ratio	1.50
Lysine	0.82
Meth + Cystiene	0.66
ME: kcal/kg	2500.1

The proximate composition of Rauvolfia vomitoria root is presented in Table 2. The sample contained dry matter, crude protein, crude fibre, ash and carbohydrates at 90.08 %, 10.04 %, 17.11 %, 1.33 %, 14.57 % and 51.33 g/100g respectively. The crude protein obtained in Rauvolfia vomitoria root is lower than the values reported for Pentadiplandra brazzeama (11.30 %), Piper umbellatum (11.30 %), Scoradophleus zenkeri (12.2 %) and Solanum melongena (14.0 %) reported by Armand et al. (2012). However, the protein value in Rauvolfia vomitoria is low, it is an indication that it cannot be used as a protein supplement in the diet of animals (NRC, 1994). Crude fibre value obtained was lower than the values reported for Momordica charantia (51.38 %) and Morinda lucida stem bark (53.49 %) reported by Olanipekun et al. (2016), but in agreement with the reports of Chinelo and Ujunwa (2017) who reported a value of 17.00 % in Boerhavia erecta roots. According to Alagbe (2020), ash is an index used to determine the mineral content in a sample. The ash and ether extract values obtained in this study are in conformity with the values obtained by Chinelo and Ujunwa (2017) for Boerhavia diffusa leaves (14.56 %). The result on ash is a clear indication that the sample contains enough minerals for the activities of enzymes, regulation of water, electrolyte and acid-base balance in the body, as well as responsible for nerve action and functioning of the muscles (Akintayo and Alagbe, 2020). Total carbohydrate value obtained is higher than the values for Lepidium sativum (19.5 g/100g), Salvia officinalis (33.0 g/100g), Thymus capitatus (50.7 g/100g) and Hibiscus sabdariffa (19.5 g/100g) but lower than values of Corianderum sativum (63.0 g/100g) and Trigonella foenum-graecum (54.2 g/100g) reported by Ereifej et al. (2012)

Table 2: Proximate composition of Rauvolfia vomitoria root

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Parameters	% composition
Dry matter	90.08
Crude protein	10.04
Crude fibre	17.11
Ether extract	1.33
Ash	14.57
Carbohydrate (g/100g)	51.33

Phytochemical composition of Rauvolfia vomitoria root is presented in Table 3. The phytochemical result contains alkaloids (1.41 %), flavonoids (4.83 %), tannins (0.86 %), terpenoids (0.18 %), saponins (0.51 %), phenols (0.71 %), steroids (0.05 %) and anthraquinone (0.10 %). In order of abundance flavonoids > alkaloids > tannins > phenols > saponins > terpenoids > anthraquinone > steroids respectively. The result obtained are in conformity with the values obtained by Chinelo and Ujunwa (2017); Jumuna et al. (2014) and Labaran et al. (2016). Phytochemicals are natural bioactive compounds that are derived from plants and incorporated into livestock feed to enhance productivity (Gadde et al., 2017). The main bioactive compounds of phytochemicals are polyphenols and their composition and concentration vary according to geographical location, parts of the plant, harvesting season, storage conditions, processing methods and environmental factors (Gadde et al., 2017; Olafadehan et al., 2020). This bioactive chemical confers plants the ability to perform multiple biological activities such as: anti-inflammatory, antiviral, antifungal, hypolipidemic, antioxidants, anti-allergic, hepato-protective and neuro-protective. However, the values were below lethal dose levels reported by Alagbe and Oluwafemi (2019) who evaluated the effects of feeding different levels of Indigofera zollingeriana to growing rabbits.

Table 3: Phytochemical composition of Rauvolfia vomitoria root

Constituents	% composition
Alkaloids	1.41
Flavonoids	4.83
Tannins	0.86
Terpenoids	0.18
Saponins	0.51
Phenols	0.71
Steroids	0.05
Anthraquinone	0.10

Table 4 shows the vitamin composition of Rauvolfia vomitoria root. The sample contains β-carotene, vitamin B1, vitamin B2, vitamin B3, vitamin B12, vitamin C, vitamin D, vitamin E and vitamin K at 1.73mg/100g, 18.11 mg/100g, 0.33 mg/100g, 0.25 mg/100g, 0.30 mg/100g, 41.03mg/100g, 0.10 mg/100g, 0.41 mg/100g and 0.12 mg/100g respectively. In order of abundance vitamin C > vitamin B1 > β-carotene > vitamin E > vitamin B2 > vitamin B12 > vitamin B3 > vitamin K > vitamin D. Vitamins are direly important for human health, growth, development, reproduction and maintenance,

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and their deficiencies are imposing serious health hazards (Mohammad et al., 2017). They are diverse in nature relative to fats, carbohydrates and proteins and differentiated from other groups by their organic nature and their classification depends on chemical nature and function (Asensi-Fabado et al., 2010). The high concentration of vitamin C is an indication that the sample will effectively perform the role of antioxidants, thus scavenging free radicals (Leskova et al., 2006; Alagbe et al., 2020). Vitamin B1 is essential for the normal functioning of the nervous system, digestive system and brain (Keogh et al., 2012). β-carotene are precursors of vitamin A and the provide animals with good vision, support to immune system and inflammatory systems, cell growth and development, antioxidant activity, promoting proper cell communication (WHFoods, 2017). Vitamin E protects the membrane fats from oxidative damage and maintains the cellular functioning. This vitamin also protects the food from oxidative damage during storage and processing (WHFoods, 2017). Vitamin B2 provides antioxidative protection and promotes iron metabolism in the body (Lanska, 2010). B12 vitamin also plays important role in energy metabolism and other biological processes while B3 is responsible for antioxidative defense (Youdim et al., 2012; Alagbe and Oluwafemi, 2019). Vitamin K is key in blood clotting while vitamin D is important for normal body functioning (Wagner and Greer, 2008; Shreer and Newman, 2012; Shreer et al., 2012).

Table 4: Vitamin composition of Rauvolfia vomitoria root

Parameters	Composition (mg/100g)
β-carotene	1.73
Vitamin B1	18.11
Vitamin B2	0.33
Vitamin B3	0.25
Vitamin B12 **Vitamin C**	0.30 41.03
Vitamin D	0.10
Vitamin E	0.41
Vitamin K	0.12

Table 5 reveals the performance characteristics of growing rabbits fed different levels of Rauvolfia vomitoria root. Initial body weight (IBW), final body weight (FBW), final weight gain (FWG) and average daily weight gain (ADWG) ranges between 530.1 – 533.0 g, 997.6 – 1331.6 g, 466.7 – 801.5 g and 5.59 – 9.51 g respectively. IBW, FBW, FWG and ADWG were highest in T4, T5, intermediate in T2, T3 and lowest in T1 ($P<0.05$). Total feed intake (TFI), average daily feed intake (ADFI), feed conversion ratio (FCR) ranged between 8800.2 – 8918.0 g, 104.8 – 106.2 g and 5.99 – 9.34 respectively. TFI increased from T1 to T5, though not at a significant rate ($P>0.05$). FCR and mortality were significantly different among the treatment ($P<0.05$). Highest mortality was recorded in T1 (1.10 %), none was recorded in the other treatments. The results on weight gain in this study are in close agreement with the Ogbuewu et al. (2010); Abu at al. (2016) when growing rabbits were fed diets containing foliage of browse trees. Higher weight gain in rabbits fed T4 and T5 could be attributed to the presence of phytochemicals in Rauvolfia vomitoria root. According to Oluwafemi et al. (2020); Caspar (2002), bioactive chemicals in feed is capable of promoting flavor, secreting of digestive fluids and total feed intake, thus promoting better performance. Medicinal plants could also act as modifiers of metabolic activities (El-Ghalid et al., 2019). According to Huyghebaert et al. (2011); Humphrey and Klasing (2003), herbal plants are capable of performing antimicrobial role, reducing growth depressing metabolites and inhibiting the production and excretion of cytokines by immune cells, this could be the reason why mortality was not recorded in T2, T3, T4 and T5.

Table 5: Performance characteristics of rabbits fed different levels of Rauvolfia vomitoria root

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Parameters	T1	T2	T3	T4	T5	SEM
IBW (g)	530.9	531.8	533.0	532.3	530.1	0.24
FBW (g)	997.6^{c}	1190.3^{b}	1221.0^{a}	1223.4^{a}	1331.6^{a}	5.61
FWG (g)	466.7^{c}	658.5^{b}	688.0^{b}	691.1^{b}	801.5^{a}	2.04
ADWG (g)	5.59^{c}	7.84^{c}	8.20^{b}	8.23^{b}	9.51^{a}	0.03
TFI (g)	8800.2	8900.1	8910.2	8913.0	8918.0	8.12
ADFI (g)	104.8	106.0	106.0	106.1	106.2	1.20
FCR	9.34^{a}	7.33^{b}	7.72^{b}	7.75^{b}	5.99^{c}	0.88
MORTALITY	1.10	-	-	-	-	0.01

IBW: Initial body weight; FBW: final body weight; FWG: final weight gain; ADWG: Average daily weight gain; TFI: total feed intake; ADFI: Average daily feed intake; FCR: feed conversion ratio; SEM: Standard error of mean.

Caecal microbial population of weaner rabbits fed different levels of Rauvolfia vomitoria root is presented in Table 6. Escherichia coli and lactobacilli count ranged between 25.01 – 42.10 (Cfu/g) and 27.88 – 50.02 (Cfu/g) respectively. E. coli and lactobacilli count were significantly ($P<0.05$) different among the treatments. According to Wong et al. (2008), various phytochemicals exhibit a wide spectrum of antibacterial activities against Gram – positive and Gram – negative bacteria. Bioactive chemicals in plants are also capable of eliminating pathogenic organisms via: direct production antagonism via production of antimicrobials, competitive exclusion through occupation of specific binding sites, stimulation of the immune response resulting in host – exclusion of the pathogen, competition for nutrients that limit microbial growth and enhanced gut health through restoration of epithelial integrity (Toole and Cooney, 2008). Flavonoids are capable of acting as an antifungal and antioxidants, thus scavenging free radicals and preventing diseases (Saleem et al., 2005; William et al., 2004; Alagbe, 2017). Tannins are known to possess both antibacterial and anti-viral activity (Adisa et al., 2010). Alkaloids are the most efficient therapeutically significant plant substances commonly found to have antimicrobial properties due to their ability to intercalate DNA of the microorganisms (Kasolo et al., 2010). Phenols are strong antioxidants while saponins are involved in anti-inflammatory activities (Hassan et al., 2012; Hollman, 2001). However, the results obtained in this study is in agreement with the findings of Khan et al. (2012); Kamel (2001); Ishihara et al. (2001) who examined the improvement of intestinal microflora balance in animals by green tea extracts.

Table 6: Caecal microbial population of weaner rabbits fed different levels of Rauvolfia vomitoria root

Parameters (Cfu/g)	T1	T2	T3	T4	T5	SEM
Escherichia coli	42.10^{a}	31.04^{b}	28.33^{c}	25.54^{c}	25.01^{c}	0.08
Lactobacilli	27.88^{c}	33.90^{c}	40.08^{b}	48.21^{b}	50.02^{a}	0.02

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CONCLUSION

The use of Rauvolfia vomitoria root is capable of promoting food safety and preventing various ailments in human and animals. It is a cheaper alternative because it contains several bioactive chemicals such as alkaloids, flavonoids, glycosides, tannins, terpenoids, phenols etc. These constituents at below lethal dose are considered safe, effective and relatively cheap. Rauvolfia vomitoria root extract have proven to perform multiple biological roles especially when fed to weaner rabbits at 80 ml/ liter concentration. It was concluded that feeding animals at this concentration enhanced feed intake, growth performance and was able to suppress the activities of pathogenic bacteria.

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