International Journal of
Biological Engineering and Agriculture

ISSN: 2833-5376
Volume 1 | No 1 | Jul-2022

Growth Performance, Semen Quality Characteristics and Hormonal Profile of Male Rabbit Bucks Fed *Rubia Cordifolia* Root Extracts

Alagbe. J. O.[1], Shittu. M. D.[2], Ramalan. S. N. [3], Tanimomo. K. B[4] and Adekunle, David Ajagbe[5]

[1]Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India

[2]Department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomosho, Oyo State

[3]Veterinary Teaching Hospital, University of Abuja, Nigeria

[4]Department of Animal Production and Health, Faculty of Veterinary Medicine, University of Abuja, Nigeria

[5]Department of Animal Production, Kogi State University Anyigba, Nigeria

***Corresponding Author: Alagbe, J.O; dralagbe@outlook.com; Orcid no: 0000 0003 0853 6144**

Abstract: 32 – 7 weeks weaned male rabbits (Newzealand × Chinchilla) weighing 611.3 ± 10 g were randomly distributed into 4 groups of 8 rabbits per treatment with one animal per replicate in a completely randomized design. Rabbits in treatment 1 (control) designated as T1 was fed basal diet with 0 mL *Rubia cordifolia* root extracts (RCE) while T2, T3 and T4 were fed basal diet with 20 mL, 40 mL and 60 mL per litre of water/day. Basal diet was formulated to meet the nutrient requirements of growing rabbits according to the recommendation of National Research Council (NRC, 1977). The experiment lasted for 12 weeks during which strict biosecurity measures were observed. Feed and water were also given *ad libitum*. Gas chromatography mass spectrometry of *Rubia cordifolia* root extracts reveals the presence of 21 bioactive compounds which accounts for 92.46 %. 9-Octadecenoic acid had the highest concentration (29.16 %) while 4-Methoxy-2-nitroformanilide had the lowest concentration (0.02 %). Average body weight gain (ADWG) and feed conversion ratio of rabbits in T4 were better (P<0.05) compared to the other treatments. Average daily feed intake (ADFI) in T1 was similar to T2 and T3 but slightly higher than T4 (P>0.05). Highest mortality was recorded in T1 (2.51 %) followed by T2 (0.05 %) none was recorded in T3 and T4 (P<0.05). Testosterone, luteinizing hormone and thyroid stimulating hormone values were significantly (P<0.05) influenced by the treatments while follicle stimulating hormone were not significantly (P>0.05) different among the treatments. Semen results showed a significant (P<0.05) decrease in sperm concentration, live sperm and motility with a significant (P<0.05) increase in abnormal sperm compared to the other treatments. It can be concluded that *Rubia cordifolia* root extracts has bioactive compounds with therapeutic properties and could be tolerated by rabbit bucks up to 60 mL per litre without causing any negative effect on the general health and performance of animals.

Keywords: *Rubia cordifolia* root extracts, performance, phytochemicals, hormones, semen

Introduction

Plant derived additives have been identified to contain bioactive compounds with effects similar to antibiotic growth promoters in three main areas, i.e., gut micro-flora, antioxidant properties, and liver function without compromising intestinal health and/or the bird's genetic potential (Hernandez, *et al*., 2004; Singh *et al*., 2021). The addition of plants and their extracts into diets is aimed at improving the productivity of livestock through amelioration of undesired feed properties, promotion of the animal's production performance and improving the quality of food derived from those animals (Kolodziej-Skalska *et al*., 2011; Agubosi *et al*., 2022; Shittu *et al*., 2021). Herbs such as herbs, spices, and various plant extracts have received increased attention as possible antibiotic growth promoters (Kim *et al*., 2009; Alagbe and Ushie, 2022; Oluwafemi *et al*., 2021).

Phytochemicals come from natural sources and are generally recognised as safe (GRAS) which make them good candidates to be used as feed additives in livestock production in comparison with antibiotics (Windisch *et al*., 2008; Alagbe, 2017). The biological mechanism of action of phytochemicals depends on their chemical structure (Kim *et al*., 2010; Alagbe *et al*., 2022). Phytochemicals used as poultry feed additives can improve animal's health and performance because of their anti-microbial, anti-bacterial, anti-helminthic and anti-stress (Peric *et al*., 2009; Shittu *et al*., 2022) and anti-oxidant properties (Applegate *et al*, 2010; Alagbe *et al*., 2021), and their ability to modulate gut microbiota (Donoghue, 2009; Alagbe *et al*., 2021) and enhance immune responses (Kim *et al*., 2008; Adewale *et al*., 2021). Environment factors such as nutrition, temperature, humidity, seasonal changes and animal management (health and housing) have a measurable effect on semen quality (Cross, 1998). Sperm morphology, motility, sperm concentration and volume per ejaculate are common criteria for evaluating semen quality (Ademola, 2003).

Rubia cordifolia also known as Indian madder belongs to the family Rubiaceae is a perennial flowering plant widely distributed in Asia (Bhatt and Kushwah, 2013). The stem, root and leaves of *Rubia cordifolia* is traditionally used for the treatment of gastro-intestinal disorder, skin disease, malaria, typhoid, diabetes, liver diseases, menstrual and urinary diseases, cancer, inflammations, pneumonia, cough, chronic bronchitis, hemoptysis and other viral infections (Bhat *et al*., 2018; Prajapati and Parmar, 2011; Musa *et al*., 2020; Alagbe, 2017).

Several studies have demonstrated the anti-bacterial, antimicrobial, hepato-protective, antiviral, anti-rheumatic, immunomodulatory, cytotoxic, anti-ulcer, anti-fibrotic, antioxidant, anti-proliferative, antifungal and anti-helmithic properties in *Rubia cordifolia* extract (Boldizs *et al*., 2006; Kalyoncu *et al*., 2006; Gao *et al*., 2003; Alagbe, 2020). Aqueous *Rubia cordifolia* root, leaf and stem bark extract have proven to suppress the activities of some pathogenic bacteria such as: *Salmonella spp*, *Staphyllococcus pyogenes*, *Staphyllococcus aureus*, *Bacteriodes spp* and *E. coli* in animals (Ino *et al*., 1995; Kamuhabwa *et al*., 2000; Wei *et al*., 2011).

In view of these abundant potentials in *Rubia cordifolia*, this experiment was designed examine the growth performance, semen quality characteristics and hormonal profile of male rabbit bucks fed *Rubia cordifolia* root extracts.

Materials and Methods

Site of the experiment

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Institute, Gujarat, India with a coastline of 1,600 Km, 23^{o} 13'N 72^{o}41'E.

Collection, preparation and gas chromatography mass spectrometry (GC-MS) of *Rubia cordifolia* root extract (RCE)

Rubia cordifolia root was collected from Sumitra Research Institute in the month of April 2021. It was identified and authenticated by a certified taxonomist Dr. Xing Liu at the Department of Biological Sciences. Samples of *Rubia cordifolia* root were thoroughly washed with running tap water and air dried for 2 weeks to obtain a constant weight. Dried *Rubia cordifolia* root was pounded into powder with a mortar and pestle it was thereafter stored in a well labeled container.

Two hundred grams of dried *Rubia cordifolia* root powder was soaked in 1 liter for 72 hours, kept in the refrigerator at 4^{o}C and stirred at 3 times daily using a spatula. The sample was filtered via a Whatman's No.1 filter paper (10 cm). Thereafter, the filterate was stored in a laboratory labeled container and sent to the laboratory for further analysis.

Gas chromatography mass spectrometry (GC-MS) analysis of *Rubia cordifolia* root extracts (RCE) was carried out with Varian 450 GC system (Model 1100 series, China) with temperature and pressure range of 50^{o}C to 450 ^{o}C isothermal 1079 PTV injector and 0 to 100 psi, consisting of splitless injector with total flow of 500 mL/minutes at 10 psi. The introduced sample (RCE) was passed through a rapid column at a cool down rate of 40 ^{o}C to 50 ^{o}C at 4.5 minutes with an electron range of 150eV. Bioactive compounds were identified with standard compounds in National Institute of Standard and Technology (NIST).

Animal management, diet formulation and experimental set-up

32 – 7 weeks weaned male rabbits (Newzealand × Chinchilla) weighing 611.3 ± 10 g were purchased from a reputable commercial breeding farms in Gujarat india and housed individually in an all wired galvanized cage measuring 50 cm × 50 cm × 30 cm: length × width × height suspended 120 cm above the ground, equipped with automatic nipple drinker and a metallic manual clay feeder was kept in each cage. Before the arrival of the animals, cages and pens were properly disinfected with Cid 2000 at10 mL per 20 liters of water. On arrival, rabbits were given anti-stress and randomly distributed into 4 groups of 8 rabbits per treatment with one animal per replicate in a completely randomized design. Rabbits were acclimatized before the commencement of the experiment during which they were given prophylactic treatment against parasites (endo and ecto-parasites) with ivermectin injection and bacterial infections (Oxytrox®) strictly adhering to the manufacturers recommendation on drug administration.

Basal diet was formulated to meet the nutrient requirements of growing rabbits according to the recommendation of National Research Council (NRC, 1977) as presented in Table A. Rabbits in treatment 1 (T1) was fed basal diet with 0 mL *Rubia cordifolia* root extracts (RCE) while T2, T3 and T4 were fed basal diet with 20 mL, 40 mL and 60 mL per litre of water/day. The experiment lasted for 12 weeks during which strict biosecurity measures were observed. Feed and water were also given *ad libitum*.

Performance traits

Daily feed intake (g)

Daily feed intake was calculated by subtracting feed served from left over. It can be expressed as:

Daily feed intake (g) = Feed served (g) – Feed left over (g)

Body weight gain (g)

Body weight gain was calculated by subtracting final body weight from initial body weight

Body weight gain (g) = Final body weight (g) – Initial weight gain (g)

Feed conversion ratio (g)

Feed conversion ratio was calculated by dividing feed consumed by body weight gain as expressed below:

Feed conversion ratio (g) = Feed consumed (g)

Body weight gain (g)

Mortality was recorded as it occurs

Hormonal evaluation

Blood samples were collected from the marginal ear veins into vacutainer bottles without ethylene diamine tetra acetic acid from 4 randomly selected rabbits per treatment for hormonal assay using commercial diagnostic kit (AIA-360 Automated Immunoassay analyzer, USA) with dimension 1016 mm × 665 mm × 762 mm (width × depth × height).

Semen collection and evaluation

A 2-week period was used to train the bucks for semen collection. Semen was finally collected from the buck using the artificial vagina (AV) described by Herbert and Adejumo (1995). Prior to semen collection, the AV was warmed for a few minutes in warm water at a temperature slightly above body temperature and thereafter drained. Semen collection was done between 7.00 and 9.00 am to ensure that optimum quality semen were obtained.

The semen was promptly assessed for semen quality parameters such as semen colour, semen volume, mass activity, sperm motility, sperm concentration and percentage live sperm using Computer Assisted Semen Analyzer Bonraybio (Taichung City, Taiwan).

Statistical analysis

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (23.0) and significant means were separated using Duncan's test of the same statistical package.

The model: $Dij = \mu + \alpha i + \beta ij$ was used in this experiment:

Where Dij = any of the response variables; i = the overall mean; αi = effect of the xth treatment and βij = random error due to experimentation

Table 1: Chemical composition of basal diet

Ingredient		Quantity (Kg)
Maize		21.0
Wheat offal		35.0
Palm kernel meal		15.95
Groundnut cake		9.00
Soyabean meal		12.65
Bone meal		1.60
Limestone		1.00
Fish meal (65 %)		1.00
Salt		0.30
*Vitamins and minerals mixture		0.20
Rice offal		2.00
Methionine		0.10
Lysine **Total**		0.20 **100.0**
Chemical analysis		(% DM)
Dry matter		89.31
Ash		6.60
Crude protein		15.21
Crude fibre		14.26
Ether extract		2.34
Nitrogen free extract		51.80
Digestible energy (Kcal/kg)		2584

*Each 1 kg contains: 10000 IU vitamin A, 2680 IU vitamin D_3, 10 IU vitamin E, 2.68 mg vitamin K, 10.68 mg calcium pantothenate, 0.022 mg vitamin B_{12}, 0.668 mg folic acid, 400 mg choline chloride, 26.68 mg chlorotetracycline, manganese 133.34 mg, 66.68 mg iron, 53.34 mg zinc, 3.2 mg copper, 1.86 mg iodine, 0.268 mg cobalt, 0.108 mg selenium.

Results and Discussion

Bioactive compounds in *Rubia cordifolia* root extracts using GC-MS analysis

Medicinal plants contain several of bioactive compounds, such as phenolics, flavonoids, terpenoids, carotenoids, saponins and alkaloids in their stems, leaves, roots, seeds, flowers and twigs (Adewale et al., 2021). These compounds are widely used in the food, cosmetic and pharmaceutical industries because they possess; antioxidant (Shittu *et al*., 2020), antimicrobial (Singh et al., 2021), anti-inflammatory (Agubosi et al., 2022), hepato-protective (Olafadehan et al., 2021), antifungal and antiviral (Oluwafemi et al., 2020), immune-modulatory, cytotoxic, hypolipidemic, antibacterial, anti-tumor, antipyretic, antiplasmodial, antifibrotic (Alagbe et al., 2022) and antiproliferative properties (Agubosi et al., 2022). Gas chromatography mass spectrometry of *Rubia cordifolia* root extracts reveals the presence of 21 bioactive compounds which accounts for 92.46 %. 3-deoxy-d-mannoic acid contains (0.44 %), 4-Methoxy-2-nitroformanilide (0.02 %), γ-terpinene (1.10 %), β-fenchol (0.40 %), 3-Allyl-6-methoxyphenol (1.67 %), Glycidol stearate (0.10 %), 2-Methyl -4-vinylphenol (2.05 %), α-cubebene (2.09 %), Carbonic acid (0.77 %), 9,12-Octadecanoic acid (25.06 %), α-longipinene (0.75 %), Terpinen-4-ol (1.04 %), 1,3 propanediol, 2-ethyl 2-hydroxymethyl (14.71 %), γ-terpinene (0.94 %), γ-eudesmol (1.13 %), 9-Octadecenoic acid (29.16 %), Torreyol-α-cadinol (0.07 %), 1,2-Cyclopentanedione (0.30 %), Ethylene diacrylate (0.50 %) and 4-methyl-2,3-hexadien -1-ol (11.20 %). 9-Octadecenoic acid had the highest concentration while 4-Methoxy-2-nitroformanilide had the lowest concentration. The result obtained in this study agrees with the findings of Mohammad et al. (2018); Kaur et al. (2008); Singh et al. (2021). A synergistic combination of these bioactive compounds allows *Rubia cordifolia* root extracts to be used in the treatment of various ailments due to their therapeutic properties (Bsau *et al*., 2005; Gupta *et al*., 2011); Raghad and Abdul (2017).

Table 2: Bioactive compounds in *Rubia cordifolia* root extracts using GC-MS analysis

Compounds	Area (%)	R.T (min)	Mole. wgt	Functions
3-deoxy-d-mannoic acid	0.44	10.78	162	Antimicrobaial and antioxidant
4-Methoxy-2-nitroformanilide	0.02	13.09	196	Antifungal
γ-terpinene	1.10	9.43	188	Hepatoprotective and antifungal
β-fenchol	0.40	10.70	201	Anti-inflammatory, antibacterial and analgesics
3-Allyl-6-methoxyphenol	1.67	8.33	164	Antiprotozoal and cytotoxic
Glycidol stearate	0.10	10.56	340	Anti-androgenic, antiviral and anti-inflammatory
2-Methyl -4-vinylphenol	2.05	12.74	150	Hepato-protective, hypolipidemic, antimicrobial and antioxidant
α-cubebene	2.09	18.10	102	Antibacterial, antifungal, angelsics antipyretic and antioxidant
Carbonic acid	0.77	22.60	228	Antiviral and antioxidant
9,12-Octadecanoic acid	25.06	18.35	280	Cytotoxic, antioxidant, anti-inflammatory, antitumor, antifungal
α-longipinene	0.75	19.22	64	Anti-inflammatory, antioxidant, anti-depressant and antifungal
Terpinen-4-ol	1.04	14.30	87	Anti-fibrotic, anti-inflammatory and hypolipidemic
1,3 propanediol, 2-ethyl 2-hydroxymethyl	14.71	20.21	95	Antibacterial, anti-inflammatory, antipyretic, antihelminthic and antifungal
γ-terpinene	0.94	21.38	71	Amtioxidant and anti-

				inflammatory
γ-eudesmol	1.13	30.93	48	Cytotoxic and hepato-protective
9-Octadecenoic acid	29.16	29.51	280	Antifungal
Torreyol-α-cadinol	0.07	17.25	52	Anitiviral, hepato-protective and antioxidant
1,2-Cyclopentanedione	0.30	29.00	98	Anti-microbial, anti-proliferative, antiviral, antihelminthic and antibacterial
Ethylene diacrylate	0.50	14.20	170	Analgesics, antibacterial, antifungal
4-methyl-2,3-hexadien -1-ol	11.20	28.10	166	Antioxidant, anti-proliferative, antifungal and anti-inflammatory
Total	92.46			

R.T: reaction time (minutes)

Growth performance of rabbits fed different levels of *Rubia cordifolia* root extracts (RCE)

Growth performance of rabbits fed different levels of *Rubia cordifolia* root extracts (RCE) is presented in Table 3. Initial body weight (IBW), final body weight (FBW), weight gain (WG), average daily weight gain (ADWG), total feed intake (TFI), average daily feed intake (ADFI) and feed conversion ratio (FCR) ranged from 611.3 – 619.5 g, 1900.8 – 2300.4 g, 1282.1 – 1680.9 g, 8652.1 – 9103.1 g, 103.0 – 108.4 g, 4.00 – 4.84, 0.50 – 2.51 % respectively. ADWG value was highest in T4, intermediate in T2 and T3 and lowest in T1 (P<0.05). Conversely, FCR value was maximum in T1, intermediate in T2 and T3 and lowest in T4 (P<0.05). ADFI were not significantly (P>0.05) affected among the treatments. Highest mortality rate was recorded in T1 (2.51 %) followed by T2 (0.50 %) while none were recorded in the other treatments (P<0.05). Higher ADWG recorded among rabbits fed different levels of *Rubia cordifolia* root extract (RCE) indicates that the test ingredients enhanced nutrient utilization by stimulating the activities of enzymes and preventing dysbiosis due to the presence of several bioactive compounds (Table 2) thus enhancing performance (Sandip *et al.*, 2022; Muritala *et al.*, 2022). The result obtained in this study agrees with the findings of Ogbuewu *et al.* (2010); Oluwfemi and Alagbe (2019). The enhanced nutrient digestibility consequently enhances feed intake and health status of rabbits (Shittu *et al.*, 2021). RCE also posses antioxidant and immune-modulatory properties due to the presence of α-cubebene, 3-deoxy-d-mannoic acid, Torreyol-α-cadinol, α-longipinene, 2-Methyl -4-vinylphenol and β-fenchol (Topala *et al.*, 2014; Kokila *et al.*, 2016).

Table 3: Growth performance of rabbits fed different levels of *Rubia cordifolia* root extract (RCE)

Parameters	Control (T1)	T2	T3	T4	SEM	LOS
IBW (g)	618.7	611.3	617.6	619.5	5.07	Ns
FBW (g)	1900.8^{c}	2022.8^{b}	2028.6^{b}	2300.4^{a}	20.80	*
WG (g)	1282.1^{c}	1411.5^{b}	1411.0^{b}	1680.9^{a}	9.33	*
ADWG (g/d)	15.26^{c}	16.80^{b}	16.80^{b}	20.01^{a}	0.90	*
TFI (g)	8652.1	8800.3	8872.1	9103.1	35.21	Ns
ADFI (g)	103.0	105.0	106.0	108.4	1.50	Ns
FCR	4.84^{a}	4.18^{b}	4.10^{b}	4.00^{c}	0.02	*
MOR (%)	2.51^{a}	0.50^{b}	-	-	0.001	*

Means in the same row not sharing same superscript are significantly ($P<0.05$) different

IBW: initial body weight; FBW: final body weight; WG: weight gain; ADWG: average daily weight gain; TFI: total feed intake; ADFI: Average daily feed intake; MOR: mortality; * Significant; Ns: non-significant; LOS: level of significant; T1: basal diet + 0 mL RCE; T2: basal diet + 20 mL RCE/day; basal diet + 40 mL RCE/day; basal diet + 60 mL RCE/day.

Hormonal profile of rabbits fed different levels of *Rubia cordifolia* root extracts (RCE)

Table 4 shows the hormonal profile of rabbits fed different levels of *Rubia cordifolia* root extracts (RCE). The hormones determined includes: testosterone (TES), follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH) which ranged from 2.06 – 4.00 (I.U/L), 6.50 – 6.98 (I.U/L), 8.80 – 12.30(I.U/L) and 0.93 – 2.03 (I.U/L) respectively. TES, LH and TSH values were significantly (P<0.05) influenced among the treatments. The values follow similar pattern and were highest in T4, intermediate in T2 and T3 and lowest in T1. This is a clear indication that the bioactive compounds in *Rubia cordifolia* root extract (RCE) is capable of activating the activities gonadotropin releasing hormone which stimulates the secretion of LH, which in turns stimulates gonadal secretion of testosterone, estrogen and progesterone. Conversely, FSH values were not significantly (P>0.05) different among the groups. The result obtained in this study agrees with the findings of Olatundun and Ogunlade (2020). According to Amao *et al*. (2013); Brucker *et al*. (1998), FSH and LH are secreted from the anterior pituitary cells of animals (gonadotrophs) with the aim of stimulating the gonads - in males, the testes and in females, the ovaries. Diminished secretion of LH or FSH can result in failure of gonadal function (hypogonadism), thus leading to poor sperm cell production (Ahemen *et al*., 2013). FSH plays for sperm production. It supports the function of Sertoli cells, which in turn support many aspects of sperm cell maturation (Ghassabian and Trasande, 2018; Kay, 2014). Kilgour *et al.* (1984); Jiang (2007) reported that FSH is necessary for the establishment of the normal population of Sertoli cell and the stimulation of the production of androgen-binding protein from the Sertoli cells. Androgen-binding protein binds with the testosterone making it available for its function in spermatogenesis (Bearden and Fuquay, 1997; Lovecamp and Devis, 2003). Testosterone is responsible in maintaining optimum conditions for spermiogenesis, spermatozoa transport and semen deposition near the site of fertilisation in the female (Kay, 2014; Castellini, 2003).

Table 4: Hormonal profile of rabbits fed different levels of *Rubia cordifolia* root extracts (RCE)

Parameters	T1	T2	T3	T4	SEM	LOS
TES (I.U/L)	2.06[c]	3.49[b]	3.55[b]	4.00[a]	0.02	*
FSH (I.U/L)	6.50	6.78	6.93	6.98	0.06	Ns
LH (I.U/L)	8.80[c]	10.10[b]	12.09[a]	12.30[a]	0.15	*
TSH (I.U/L)	0.93[c]	1.12[b]	1.15[b]	2.03[a]	0.01	*

* Significant; Ns: non-significant; LOS: level of significant; T1: basal diet + 0 mL RCE; T2: basal diet + 20 mL RCE/day; basal diet + 40 mL RCE/day; basal diet + 60 mL RCE/day; TES: testosterone; FSH: follicle stimulating hormone; LH: luteinizing hormone; Thyroid stimulating hormone

Semen parameters of rabbit buck fed different levels of *Rubia cordifolia* root extracts (RCE)

Semen parameters of rabbit buck fed different levels of *Rubia cordifolia* root extracts (RCE) is presented in Table 5. The semen colour was milky across the treatment while sperm volume, semen pH, sperm concentration, live sperm percentage, abnormal sperm percentage and motility percentage ranged from 0.51 – 0.66 mL, 7.00 – 7.18, 21.60 – 32.34 ($\times 10^6$/mL), 75.12 – 84.12 %, 10.04 – 14.21 % and 54.18 – 70.40 % respectively. Semen pH and colour were not significantly (P>0.05) different among the treatments. Conversely, sperm volume, sperm concentration, live sperm percentage, abnormal sperm percentage and motility percentage were significantly (P<0.05) influenced by the treatment. According to Abd-Azim and El-kamash (2015) variation in semen colour, semen pH, semen density and motility could be attributed differences in breed of rabbit bucks. Daader and Saleem (2005); El-Sheikh and Saleem (2010) reported semen volume to increase with age and body weight. Sperm concentration and live sperm concentration in bucks fed T2, T3 and T4 were better (P<0.05) than those fed T1. This variation in values could be attributed to the antioxidant properties in RCE due to the presence of some secondary metabolites. Hoogenboezem and Swanepoel (2000) reported that semen quality and scrotal circumference are affected by factors related to underdevelopment of the testes and testicular degeneration. The frequency of abnormal sperm cells has been found to increase with factors such as extreme in temperature, malnutrition, toxins or antinutrients as well as activities of free radicals (Jimoh and Ewuola, 2018; Marai *et al*., 2001). This has been observed to result in lower ejaculate volume and sperm motility, increase in the percentage of abnormal sperm and a decrease in the total live sperm especially among rabbit bucks in T1 (Rathore, 1970). Underdevelopment of the testis has been reported as one of the factors that can affect the quality of the semen (Ganaie *et al*., 2013). However, the result observed in this experiment is in agreement with the findings of Ajuogu *et al*. (2018); Andrej *et al*. (2013) on the effect of herbal additive (Yucca) on rabbit spermatozoa characteristics.

Table 5: Semen parameters of rabbit buck fed different levels of *Rubia cordifolia* root extracts (RCE)

Parameters	T1	T2	T3	T4	SEM	LOS
Semen colour	Milky	Milky	Milky	Milky	-	-
Sperm volume (mL)	0.51^{b}	0.57^{b}	0.60^{a}	0.66^{a}	0.06	*
Semen pH	7.02	7.00	7.13	7.18	0.02	Ns
Sperm conc. ($\times 10^{6}$/mL)	21.60^{b}	30.21^{a}	32.18^{a}	32.34^{a}	0.24	*
Live sperm (%)	75.12^{b}	80.91^{a}	82.50^{a}	84.12^{a}	0.51	*
Abnormal sperm (%)	14.21^{a}	11.84^{b}	10.10^{b}	10.04^{b}	0.03	*
Motility (%)	54.18^{c}	68.84^{b}	70.02^{a}	70.40^{a}	0.66	*

* Significant; Ns: non-significant; LOS: level of significant; T1: basal diet + 0 mL RCE; T2: basal diet + 20 mL RCE/day; basal diet + 40 mL RCE/day; basal diet + 60 mL RCE/day

Conclusion

It was concluded that *Rubia cordifolia* root extracts (RCE) is loaded with several secondary metabolites which allows it to perform multiple biological activities such as: anti-inflammatory, antioxidant, antifungal, antivirus, antioxidant, cytotoxic, hypolipidemic, immunomodulatory etc. RCE is relative cheap, available, effective, environmentally friendly and could be tolerated by rabbit bucks up to 60 mL per litre without causing any negative effect on the general health and performance of animals.

References

1. Abd El-Azim, A and El-kamash, E. M. (2015). Evaluation of semen quality and its relation to mating system for some breeds of rabbits under environmental conditions in the middle of Egypt. Egyptian Poultry Science, 31(II), 467–480.
2. Castellini, C. (2008). Semen production and management of rabbit bucks. 9th World Rabbit Congress. June 10-13, 2008 – Verona – Italy.
3. Bearden H. J and Fuquay J. W. (1997). Applied animal reproduction. (3rd ed) Prentic- Hall. Englewood Cliff.
4. Kilgour R. J., Pisselet C. Dumbols M. P., Courot M. & Sairam M. R. (1984). Role of FSH in the establishment of spermatogenesis in the lamb .. 1*dh International Cong. Of Anim. Reprod. and AI. University of lllinios at Ubarna-Champaign, USA*, pp 42.
5. Amao E. A., Oladipo A. O. and Sokunbi O. A. (2013). Testicular Characteristics and Daily Sperm Production of Rabbit Bucks Fed Diets Containing Neem Azadirachta Indica A. Juss) Rind Meal. *Greener Journal of Agricultural Sciences.* 3(8): 623-627.
6. Ahemen T., Abu A. H. and Orakaanya T. T. (2013). Sperm quality and testicular morphometry of rabbits fed dietary levels of water spinach (Ipomoea aquatica) leaf meal. *Agriculture and Biology Journal of North America.* 21(5): 51-57.
7. Hoogenboezem, J and Swanepoel, F. (2000). Zootechnological aspect of bull fertility. *Animal Genetic Resource* Iowa. State University Press.
8. Rathore A. K. (1970). Acrosomal abnormality in ram spermatozoa due to heat stress. *British Veterinary Journal*, 126: 440-443.

9. Alagbe, J.O., Shittu, M.D and Tanimomo, Babatunde K. (2022). Influence of *Anogeissusleio carpus* stem bark on the fatty acid composition in meat of broiler chickens. *European Journal of Life Safety and Stability* 14(22): 13-22.
10. Alagbe, J.O (2022). Use of medicinal plants as a panacea to poultry production and food security: A review. *Gospodarka I Innowacje* 22(2022): 1-12.
11. Agubosi, O.C.P., Alexander, James and Alagbe, J.O. (2022). Influence of dietary inclusion of Sunflower (*Helianthus annus*) oil on growth performance and oxidative status of broiler chicks. *Central Asian Journal of Medical and Natural Sciences* 2(7): 187-195.
12. Agubosi, O.C.P., Soliu, M.B and Alagbe, J.O. (2022). Effect of dietary inclusion levels of *Moringa oleifera* oil on the growth performance and nutrient retention of broiler starter chicks. *Central Asian Journal of Theoretical and Applied Sciences* 3(3): 30-39.
13. Agubosi, O.C.P., Imudia, Favour Dumkenechukwu and Alagbe, J.O. (2022). Evaluation of the nutritional value of air dried and sun-dried sweet potato (*Ipomoea batatas*) peels. *European Journal of Life Safety and Stability* 14(22): 43-51.
14. Alagbe, J.O. (2022). *Prosopis africana* (*African mesquite*) oil as an alternative to antibiotic feed additives on broiler chickens diets: haematology and serum biochemical indices. *Central Asian Journal of Theoretical and Applied Sciences* 3(2): 19-29.
15. Agubosi, O.C.P., Wika, B.K and Alagbe, J.O. (2022). Effect of dietary inclusion of Sunflower (*Helianthus annus*) oil on the growth performance of broiler finisher chickens. *European Journal of Modern Medicine and Practice*, 2(5): 1-10.
16. Alagbe, J.O. (2022). *Prosopis africana* (*African mesquite*) oil as an alternative to antibiotic feed additives on broiler chickens diets: performance and nutrient retention. *Discovery* 58(314): 134 - 142.
17. Alagbe, J.O and Ushie, F.T. (2022). Growth performance of broiler chicks fed diets containing different levels of aqueous *Citrus aurantium* stem bark extracts. *Discovery* 58(319): 735-741.
18. Alagbe, J.O., Adedeji, M.O., Habiba, Z., Nwosu, Gloria and Wyedia Dabara Comfort (2021). Physico-chemical properties of *Indigofera zollingineriana* seed oil. *Asian Journal of Advances in Medical Science* 3(4): 306-308.
19. Agubosi, O.C.P., Oluwafemi, R.A and Alagbe, J.O. (2021). The effect of processing on the proximate, mineral and vitamin composition of Neem leaves (*Azadirachta indica*) grown in Gwagwalada, FCT, Abuja. *Abuja Journal of Agriculture and Environment*, 1(1): 293-299.
20. Adewale, A.O., Alagbe, J.O., Adeoye, Adekemi. O. (2021). Dietary Supplementation of *Rauvolfia Vomitoria* Root Extract as A Phytogenic Feed Additive in Growing Rabbit Diets: Haematology and serum biochemical indices. *International Journal of Orange Technologies*, 3(3): 1-12.
21. Daader, A. H., & Seleem, T. S. T. (2005). Response of spermatozoa of different breeds of rabbits to hypo-osmotic swelling test. In Proc: The 4th Inter. Con. on Rabbit Prod. in Hot Clim, (pp. 177–181).
22. El-Sheikh, T. M., & Seleem, T. S. T. (2010). Effect of genotype and natural or artificial insemination on indigenous and adapted rabbit performance. Scientific papers-animal science series: Lucrări Ştiinţifice - Seria Zootehnie, 65, 19–24.
23. Ganaie, A. H., Shanker, G., Bumla, N. A., Ghasura, R. S., & Mir, N. A. (2013). Biochemical and physiological changes during thermal stress in bovines. Journal of Veterinary Science and Technology, 4, 126. https://doi.org/10.4172/ 2157-7579.10001224.
24. Jimoh, O. A., & Ewuola, E. O. (2018). Thermophysiological traits in four exotic breeds of rabbit at least temperature-humidity index in humid tropics. The Journal of Basic and Applied Zoology, 79(18), 31–39. https://doi.org/10.1186/ s41936-018-0031-9.

 |

25. Marai, I.F.M., Ayat, M.S., & Abd El-Monem, U.M. (2001). Growth performance and reproductive traits at first parity of New Zealand white female rabbits as affected by heat stress and its alleviation, under Egyptian conditions. Tropical Animal Health and Production, 33:1–12.

26. Singh, A.S., Alagbe, J.O., Sharma, S., Oluwafemi, R.A and Agubosi, O.C.P. (2021). Effect of dietary supplementation of melon (*Citrallus linatus*) seed oil on the growth performance and antioxidant status of growing rabbits. *Journal of Multidimensional Research and Reviews*, 2(1): 78-95.

27. Shittu, M.D., Alagbe, J.O., Adejumo, D.O., Ademola, S.G., Abiola, A.O., Samson, B.O and Ushie, F.T. (2021). Productive Performance, Caeca Microbial Population and Immune-Modulatory Activity of Broiler Chicks Fed Different Levels *Sida Acuta* Leaf Extract in Replacement of Antibiotics. *Bioinformatics and Proteomics Open Access Journal* 5(1): 000143.

28. Alagbe, J.O (2020). Chemical evaluation of proximate, vitamin and amino acid profile of leaf, stem bark and roots of *Indigofera tinctoria*. *International Journal on Integrated Education.* 3(10): 150-157.

29. Musa, B., Alagbe, J.O., Adegbite Motunrade Betty, Omokore, E.A. (2020). Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture. *United Journal for Research and Technology*, 2(2):13-21.

30. Alagbe, J.O., Shittu, M.D and Eunice Abidemi Ojo (2020). Prospect of leaf extracts on the performance and blood profile of monogastric – A review. *International Journal of Integrated Education*. 3(7): 122-127.

31. Ghassabian A and Trasande L. (2018). "Disruption in thyroid signaling pathway: a mechanism for the effect of endocrine-disrupting chemicals on child neurodevelopment". Frontiers in Endocrinology 9 (2018): 204

32. Oluwafemi, R.A., Akinbisola, S.A and Alagbe, J.O. (2020). Nutritional and growth performance of feeding *Polylathia longifolia* Leaf Meal as partial replacement of Wheat Offal in the diet of broiler chicks. *European Journal of Biotechnology and Bioscience.* 8(4): 17-21.

33. Ogbuewu, I.P., Okoli, L.C and iloeju, M.U. (2016). Assessment of blood chemistry, weight gain and linear body measurements of pre-pubetral buck rabbits fed different levels of Neem leaf meals. *Chilean Journal of Agricultural Research*, 70(3): 515-520.

34. Kolodziej-Skalska, A., Rybarezyk, A., Matysiak, B., Jacyno, E., Pietruszka, A., Kawecka, M. (2011). Effect of dietary plant extracts mixture on pork meat quality. *Acta.Agric. Scandinavica Sec*. A61:80-85.

35. Kay, V.R. (2014). "Reproductive and developmental effects of phthalate diesters in males". Critical Review of Toxicology 44: 467- 498.

36. Hernandez, F., Madrid, J., Garcia, V., Orengo, J., and Megias, M.D. (2004). Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poultry Science*, 83:169-174.

37. Brucker-Davis F. (1998). "Effects of environmental synthetic chemicals on thyroid function". Thyroid 8: 827-856.

38. Jiang J. (2007). "Studies on developmental abnormalities in hypospadia male rats induced by maternal exposure to di-n-butyl phthalate (DBP)". Toxicology 232: 286-293.

39. Lovekamp-Swan T and Davis, B.J. (2003) "Mechanisms of phthalate ester toxicity in the female reproductive system". Environmental Health Perspective 111: 139-145.

40. Ajuogu, P.K., Herbert, U., Ibeh, M.B., Ukpabio, C.G., Onyebule, G and Akintola, A.O. (2018). Semen characteristics and testosterone levels of bucks fed Costus afer leaf. Afican Journal of Biotechnology, 17(2): 24-28.

41. Andrej, B., Martina, F., Lubica, C., Alexander, V.S and Peter, C. (2013). Effect of the herbal additive Yucca on rabbit spermatozoa characteristics. Journal of Microbiology Biotechnology and Food Sciences, 13(2): 1829-1837.

42. Applegate, T.J., Klose, V., Steiner, T. Ganner, A. and Schatzmayr, G. (2010). Probiotics and phytogenics for poultry: Myth or reality? *Journal of Applied Poultry Research*, 19: 194 – 210.

43. Kim, S.W., Fan, M.Z. and Applegate, T.J. (2008). Nonruminant Nutrition symposium on natural phytobiotics for health of young animals and poultry: Mechanisms and application. *Journal of Animal Science*, 86 (E. Supplement): E138 – E139.

44. Peric, L., Zikic, D. and Lukic, M. 2009. Application of alternative growth promoters in broiler production. *Biotechnology in Animal Husbandry*, 25 : 387 – 397.

45. Windisch, W., Schedle, K., Plitzner, C. and Kroismayr A. (2008). *Journal of Animal Science*, 86 (E. Supplement): E140–E148.

46. Donoghue, D.J. (2003). Antibiotic residues in poultry tissues and eggs: human health concerns? *Poultry Science*, 82: 618 – 621.

47. Raghad, D. H and Abdul, J. (2017). GC-MS Analysis of Extract of Rubia tinctorum having Anticancer Properties. *International Journal of Pharmacognosy and Phytochemical Research* 9(3); 286-292.

48. Boldizs´ar, I.; Sz˝ucs, Z.; F¨uzfai, Zs. and Moln´arPerl, I. (2006). Identification and quantification of the constituents of madder root by gas chromatography and high-performance liquid chromatography. *Journal of Chromatography* A, 1133: 259–274.

49. Kalyoncu, F.; Cetin, B. and Saglam, H. (2006). Antimicrobial Activity of Common Madder (Rubia tinctorum L. *Phytother. Research*, 20, 490–492.

50. Ino, N.; Tanaka, T.; Okumura, A.; Morishita, Y.; Makita, H.; Kato, Y.; Nakamura, M. and Mori, H. (1995). Acute and subacute toxicity tests of madder root, natural colorant extracted from madder (Rubia tinctorum), in (C57BL/6 X C3H)1 mice. *Toxicol Ind. Health*. 11(4):449-58.

51. Kamuhabwa, A.; Nshimo, C. and Witte, P. D. (2000). Cytotoxicity of some medicinal plant extracts used in Tanzanian traditional medicine. *Journal of Ethno-pharmacology*, 70: 143-149.

52. Gao, S.; Yu, B.P.; Li, Y.; Dong, W.G. and Luo, H.S. (2003). Antiproliferative effect of octreotide on gastric cancer cells mediated by inhibition of Akt/PKB and telomerase. W. J. G., 9(10): 2362-2365.

53. Wei, L. S.; Wendy Wee, Siong, J. Y. F. and Syamsumir, D. F. (2011). Characterization of antibacterial, antioxidant, anticancer properties and chemical composition of Malaysia Adrographis paiculata leaf extract. *Pharmacologyonline* 2: 996- 1002.

54. Olafadehan, O.A., Oluwafemi, R.A and Alagbe, J.O. (2020). Carcass quality, nutrient retention and caeca microbial population of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. *Journal of Drug Discovery*. 14(33):146-154.

55. Olafadehan, O.A., Oluwafemi, R.A and Alagbe, J.O. (2020). Performance, haemato-biochemical parameters of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. *Advances in Research and Reviews*, 2020, 1:4.

56. Prajapati SN, Parmar K.A. (2011). Anti-viral and in-vitro free radical scavenging activity of leaves of Rubia cordifolia. *International Journal of Phytomedicine*, 3:98–107.

57. Bhat, B.A, Shergojri F.A, Gaur, M, Sham, Q.J. (2018). Phytochemical analysis and in vitro antioxidant activity of rubia cordifolia. *International Journal in Recent Trends in Science Technology*, 18: 26–33.

58. Bhatt, P, Kushwah, A.S. (2013). *Rubia cordifolia* overview: a new approach to treat cardiac disorders. *International Journal of Drug Development and Research*, 5(2):47–54.

59. Kokila, T., Ramesh, P. S and Geetha, D. (2016). Biosynthesis of AgNPs using Carica Papaya peel extract and evaluation of its antioxidant and antimicrobial activities. Ecotoxicol. Environ. Saf. 134: 467–473.

60. Topală, T., Bodoki, A., Oprean, L and Oprean, R. (2014). Experimental techniques employed in the study of metal complexes-DNA–interactions. *Exp. Tech*. 62(6): 1-6.

61. Singh R, Geetanjali, Chauhan S.M. (2004). 9, 10-Anthraquinones and other biologically active compounds from the genus Rubia, *J Chem. Biodivers*, 4: 11241-1264.

62. Kaur P, Singh B, Kumar S, Kaur S. (2008). In vitro evaluation of free radical scavenging activity of Rubia cordifolia LJ. *Chinese Clinical Med*, 3:278-284.

63. Basu S, Ghosh A, Hazra B. (2005). Evaluation of the antibacterial activity of Ventilago madraspatana Gaertn, Rubia cordifolia Linn. And Lantana camara Linn. Isolation of Emodin and Physcion as active antibacterial agents. *Phytotherapeutic Research*, 19(10):888- 894.

64. Gupta V, Yadav SK, Singh D, Gupta N.(2011). International Journal of Pharmaceutical and Life Science, 2(7):952-954.

65. Alagbe, J.O., Ajagbe, A.D., Attama Jeremiah, Philemon, K.C and Bello, Kamoru, A (2020). *Albizia lebbeck* stem bark aqueous extract as alternative to antibiotic feed additives in broiler chicks diets: Haematology, Serum indices and oxidative status. *International Journal of Biological, Physical and Chemical Studies*, 2(1): 8-15.

66. Alagbe, J.O (2020). Caecal Microbial Population of Growing Grass Cutters (*Thyronoyms Swinderianus*) Fed *Phyllantus Amarus* and *Pilogstigma Thonngii* Leaf Meal Mixture as Partial Replacement for Soya Bean Meal. *Concept of Dairy and Veterinary Sciences*. 3(5): 350 – 355.

67. Muritala, Daniel Shittu., Alagbe, J.O., Ojebiyi, O.O., Ojediran, T.K and Rafiu, T.A. (2022). Growth performance and haematological and serum biochemical parameters of broiler chickens given varied concentrations of *Polyalthia longifolia* leaf extract in place of conventional antibiotics. *Animal Science and Genetics* 18(2): 57-71.

68. Alagbe, J.O. (2017). Effect of dietary inclusion of *Polyalthia longifolia* leaf meal as phytobiotic compared with antibiotics on the nutrient retention, immune response and serum biochemistry of broiler chicken. *Greener Journal of Agricultural Sciences*. 7(3):74-81.

69. Alagbe, J.O. (2017). Performance, blood profile and carcass evaluation of growing grass cutters fed diets supplemented with matured *Polyalthia longifolia* leaf meal. *Scholarly Journal of Agricultural Science*. 7(2):44-49.

70. Alagbe, J.O. (2017). Nutrient evaluation of sweet orange (*Citrus sinensis*) fruit peel as a replacement for maize in the diets of weaner grass cutters. *Scholarly Journal of Agricultural Science*. 6(8):277-282.

71. Takeli, A.L., Celik, H.R., Katlu, R and Gorgulu, M. (2006). Effect of dietary supplemental plant extracts on the performance carcass and digestive system development of broiler chicks. Proceedings of 12th European Poultry Conference, Sept. 10-14, 2006, Verona Italy.

72. Sokovic, M., Glamoclija, P.D., Marin, D and Van, L.J. (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using *in vitro* model. Molecules, 15: 7532-7546.

73. Stef, L., Dumitrescu, G and Stef, D. (2009). The effect of medicinal plants and plant extracted oils on the broiler deodunum morphology and immunological profile. Romanian Biotechnology Letter, 14: 4606-4614.

74. Umashanker, M and Shruti, M. (2011). Traditional Indian herbal medicine used as antipyretic, antiulcer, antidiabetic and anticancer: A review. International Journal of Pharmaceutical Research, 1: 1152-1159.

75. Alagbe, J.O. (2021). *Prosopis africana* stem bark as an alternative to antibiotic feed additives in broiler chicks diets: Performance and Carcass characteristics. *Journal of Multidimensional Research and Reviews*, 2(1): 64-77.

76. Alagbe, J.O. (2021). *Daniellia oliveri* leaf extracts as an alternative to antibiotic feed additives in broiler chicken diets: Meat Quality and Fatty acid composition. *Indonasian Journal of Innovation and Applied Sciences* 1(3): 177-186.