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

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ABSTRACT

32-7 weeks weaned male rabbits (New Zealand × 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: *Hormone, Phytochemicals, Rabbits, Rubia Cordifolia, Semen*

INTRODUCTION

It has been discovered that plant-derived additives contain bioactive substances with effects especially compared to those of antibiotic growth promoters in three important areas, i.e. gut microflora, antioxidant properties, and liver function, without compromising intestinal health or the genetic potential of the bird. Through the amelioration of undesirable feed qualities, stimulation of the animal's production performance, and enhancement of the quality of food obtained from those animals, the insertion of plants and their extracts into diets aims to increase the productivity of animals. Herbs including spices, herbs, and other plant extracts have received more interest as potential antibiotic growth promoters.

Phytochemicals are generally recognized as safe (GRAS) and derived from natural sources, making them preferable to antibiotics for usage as feed additives in animal production. Phytochemicals' chemical structure determines how they work biologically. Due to their anti-microbial, anti-bacterial, anti-helminthic, anti-stress, and anti-oxidant qualities along with their capacity to modify gut microbiota and boost immune responses, phytochemicals used as chicken feed additives can improve animal health and performance. Semen quality is measured in relation to environmental elements such as diet, temperature, humidity, seasonal variations, and animal management (housing and health). Sperm morphology, motility, concentration, and volume per ejaculate are often used metrics to assess the quality of semen.

A perennial blooming plant with a wide distribution in Asia, Rubia cordifolia, often known as Indian madder, is a member of the Rubiaceae family. Rubia cordifolia's stem, root, and leaves have historically been used to treat gastrointestinal disorders, skin conditions, malaria, typhoid, diabetes, liver conditions, menstrual and urinary conditions, cancer, inflammations, pneumonia, cough, chronic bronchitis, hemoptysis, and other bacterial diseases.

Numerous researches have determined that Rubia cordifolia extract possesses anti-bacterial, antimicrobial, hepatoprotective, antiviral, anti-rheumatic, immunomodulatory, cytotoxic, anti-ulcer, anti-fibrotic, antioxidant, anti-proliferative, antifungal, and anti-helminthic activities. Salmonella spp., Staphylococcus pyogenes, Staphylococcus aureus, Bacteroides spp., and E. coli have all been discovered to be inhibited by aqueous Rubia cordifolia root, leaf, and stem bark extract in animals.

Due to the abundance of potentials in Rubia cordifolia, this experiment was created to look at the hormonal profile, growth performance, and features of the semen produced by male rabbit bucks fed Rubia cordifolia root extracts.

RESEARCH METHODOLOGY

Research Location

The research was conducted at animal nutrition division, Sumitra Research Institute, India.

Collection, Preparation and Gas Chromatography Mass Spectrometry (GC-MS) of Rubia Cordifolia Root Extract (RCE)

In April 2021, Sumitra Research Institute provided rubia cordifolia root for collection. Dr. Xing Liu, a licensed taxonomist at the Department of Biological Sciences, recognized and verified it. In order to achieve consistent weight, samples of Rubia cordifolia root were carefully cleaned by clean water from the faucet and allowed to air dry for two weeks. It was then stored in a jar with clear labeling after being ground into powder from dried Rubia cordifolia root. Using a spatula, 200 grams of dried Rubia cordifolia root powder were mixed three times a day while soaking in 1 liter of water for 72 hours at 4°C. Whatman's No. 1 filter paper was used to filter the sample (10 cm). The filterate was then transferred to the lab for further examination and stored in a container with a lab label.

The analysis of Rubia cordifolia root extracts (RCE) by gas chromatography mass spectrometry (GC-MS) was performed using a Varian 450 GC system (Model 1100 series, China) with temperature and pressure ranges of 50°C to 450°C isothermal 1079 PTV injector and 0‑100 psi, consisting of split less injector with a total flow of 500 ml/minutes at 10 psi. At a cool-down rate of 40°C to 50°C in 4.5 minutes and an electron range of 150eV, the introduced sample (RCE) was sent through a quick column. Therefore at National Institute of Standard and Technology (NIST), bioactive substances were discovered alongside standard substances.

Animal Management, Diet Formulation, and Experimental Set-up

32-7 weeks weaned male rabbits (New Zealand × 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 manufacturer's 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:

$$\text{Feed conversion ratio (g)} = \frac{\text{Feed consumed (g)}}{\text{Body weight gain (g)}}$$

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 was obtained.

The semen was promptly assessed for semen quality parameters such as semen color, 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.

The Chemical Composition of Basal Diet

Ingredient	Quantity (Kg)
Maize	21.0
Wheat offal	35.0
Palm kernel meal	15.95
Groundnut cake	9.00
Soybean 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	0.20
Total	**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 chlortetracycline, 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.

RESULT 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. These compounds are widely used in the food, cosmetic and pharmaceutical industries because they possess; antioxidant (Shittu *et al*., 2020), antimicrobial, anti-inflammatory, hepato-protective, antifungal and antiviral, immune-modulatory, cytotoxic, hypolipidemic, antibacterial, anti-tumor, antipyretic, antiplasmodial, antifibrotic and antiproliferative properties. 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.

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	Antimicrobial 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, analgesics 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, anthelminthic and antifungal
γ-terpinene	0.94	21.38	71	Antioxidant 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	Antiviral, hepato-protective and antioxidant

1,2-Cyclopentanedione	0.30	29.00	98	Anti-microbial, anti-proliferative, antiviral, anthelminthic 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. 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. RCE also possess 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.

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.

Description:
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 Extract (RCE)

Based on Table 4, it 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. FSH plays for sperm production. It supports the function of Sertoli cells, which in turn support many aspects of sperm cell maturation. 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. Testosterone is responsible in maintaining optimum conditions for spermiogenesis, spermatozoa transport and semen deposition near the site of fertilization in the female.

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 color 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 color 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 color, 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 anti-nutrients as well as activities of free radicals. 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. Underdevelopment of the testis has been reported as one of the factors that can affect the quality of the semen. 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. ($\times10^{6}$/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.

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