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A Study on Changes in Gut Microflora, Blood Glucose Level and Lipid Profile of Broiler Chickens Fed with *Murraya koenigii* Supplemented Diet

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TSPJ and HADR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors RMRBM, TSPJ, HGCLG, WUNTSE and HADR managed the analyses of the study. Authors HGCLG, WUNTSE and DGY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The emergence of multiple drug resistance to human pathogenic organisms has necessitated the search for new antimicrobial substances from natural sources including plants. Also, the non communicable diseases such as diabetes mellitus and cardiovascular diseases represent an enormous, medical, social and economic burden to the public and high cost of synthetic drugs used for these diseases have become more exorbitant. As a remedial measure, attempts have been made to find alternatives with special attention to utilization of similar compounds of natural origin.

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This study was conducted to assess the effect of feeding curry leave on blood glucose level and lipid profile in broiler chicken and the antibacterial effect of curry leaves on gut microflora of broiler chicken.

Study Design: Sixty, 28 days old broiler birds were randomly allocated to four dietary treatments with three replicates of five birds per each replicate in a completely randomised design. Maize and soybean meal based control feed and three test diets prepared from the control feed by incorporating curry leaves at 0.5, 1.0 and 1.5% levels served as four dietary treatments. Feeding continued until slaughtering at 42 days of age.

Place and Duration of Study: The study was conducted in the Livestock unit of the University Farm and the sample analysis was done at the Laboratory of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka.

Methodology: Blood samples were collected at slaughter on 42nd day and lipid profile analysis (total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and Triglycerides) and blood glucose analysis was done. At sacrifice 25 g of gut content was collected aseptically from each bird for microbiological investigation and total bacterial enumeration was done. Data were analyzed using SPSS and ANOVA followed by a Tukey's post-hoc test.

Results: Serum total cholesterol level was significantly (P<0.05) lowered by 6.0%,12.4% and 15% in birds fed with 0.5%, 1% and 1.5% curry leave diets respectively compared to the control. There was no significant difference in triglycerides and HDL levels among treatments. LDL level was significantly (P< 0.05) lowered by 26.0, 30.7 and 34.6% respectively in birds fed with 0.5, 1.0 and 1.5% curry leave levels. Curry leave significantly reduced the serum glucose level by 10, 13 and 16% in birds with 0.5, 1.0 and 1.5% curry leave levels respectively. Microbiological study revealed a statistically significant reduction of gut microbes in broiler chicken. When compared to the microbial count in control ($8.9x10^8$ CFU/g**),** the count was reduced by 37.2% (5.6×10^8 CFU/g) in 1% group and by 49.1% (4.5×10^8 CFU/g) in 1.5% group. The reduction (8.5×10^8 CFU/g**)** was not significant with 0.5% curry leave level.

Conclusion: Curry leaves exerted hypoglycaemic and hypocholesterolemic effects in broiler chickens. There was ample evidence of antimicrobial effect as the inclusion levels of curry leaves increased across the diets.

Keywords: Blood glucose; broilers; cholesterol; curry leave; gut microbes.

1. INTRODUCTION

One of the major concerns of human life throughout the globe is health care. Therefore there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures, cheap, easily available with novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. In addition, big concern is the development of resistance to the antibiotics in current clinical use [1]. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [2,3]. In the present situation of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants [4,5]. Plants produce a diverse array of bioactive molecules, making them a rich source of diverse type of medicines [6,7]. Thus, natural products with pharmacological or biological activities play a very significant role in medicine [8,9]. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms [10,11]. Despite of infectious diseases, non-communicable diseases are one of the major health problems in developing and developed countries, diabetes mellitus and cardiovascular diseases represent an enormous, medical, social and economic burden to the public. Diabetes is one of the major non communicable diseases and it is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion or insulin action, or both. Many genetic and lifestyle factors are involved in the etiology of these diseases [12,13]. Although large numbers of synthetic drugs such as biguanides, sulphonylureas and thiozolidinediones [14] are being used to treat these diseases, the risk of having side effects of these drugs is a matter for consideration and also unbearable cost of those products has become a great burden to many communities at the moment [15]. As a remedial measure for this situation, attempts have been made to find other

alternatives with special attention to utilization of similar compounds of natural origin [16].

Phytochemical constituents such as alkaloids, flavonoids, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores [17]. Analysis of plant extracts curry leaves has showed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, and steroids [18]. These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms [19].

Murraya koenigii (curry leaf) belonging to the family Rutaceae is used as a leafy spice for its characterizing authentic Asian-Indian cuisine and it is used in small quantities for its distinct aroma as well as for preservation purposes. It is reported to have antioxidant, anti-diabetic, anti-carcinogenic, anti-dysenteric, stimulant, hypoglycaemic and antimicrobial activities [20,21,22]. Biologically active carbazole alkaloids are reported to have antimicrobial properties and also curry leaves have been reported to contain tocopherol, Beta carotene, lutein and alkaloids [23]. Several studies have shown the splendid health benefits of curry leaf having use in indigenous medicine in many Asian countries including India and Sri Lanka [24,25,26,27]. The chemical composition of the fresh leaves of *Murraya koenigii* consists of volatile oil. Carbazole alkaloids and triterpene have been isolated from stem bark and roots of *Murraya koenigii* [26,27]. Thus, Curry leaves merits further phytochemical, pharmacological and clinical investigations for development of an effective natural remedy to provide therapeutically effective lead compounds. The biochemicals found in Curry leaves include caryophyllene, cadinene, cadinol, Sabinene, pinene, phellandrene, terpinene, lauric acid, palmitic acid, carbazole alkaloids, bornyl acetate, humulene, ocimene and bisabolene, etc [28]. The nutrients found in curry leaves, per 100 g, include 66 g of moisture, 6 g of protein, 1 g of fat, 18 g of carbohydrate, 6 g of dietary fibre, and 4 g of minerals such as calcium, phosphorus, iron, nicotinic acid, vitamin A and vitamin C [29]. Various notable pharmacological activities of the plant such as anti-diabetic, hypocholesterolemic, anti-microbial, anti-ulcer, anti-oxidative, cytotoxic, anti-diarrhea, phagocytic and vaso-relaxing have been shown in many studies [30,31]. In addition, curry leaves also help to lower LDL cholesterol levels and increase HDL cholesterol levels, which have protective action on the heart. Combination of all these actions, help this aromatic leave to play a crucial role in controlling cholesterol and blood sugar levels in the body and thereby have a protective action of the heart and the vascular system [20,21,31,32]. Methanolic extract of curry leaves inhibited *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus uberis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Corynebacterium gravis* and *Bacillus cereus* [18]. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [33]. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell [34]. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes [35]. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. As there is a burning problem of development of resistance to the antibiotics in current clinical use [36], discovery of new antimicrobial substances from other sources including plants are vital. Studies identified several antimicrobial compounds within the curry leaves [37]. In this study effect of curry leaf on gut microbes was investigated using poultry chicken to see the possibility of using curry leaves as antibacterial agents and also study the effect of curry leave on the blood glucose and lipid profile in broiler chicken.

2. MATERIAL AND METHODS

2.1 Feeding of Curry Leave (*Murraya koenigii*)

The study was conducted in the Livestock Unit of the University Farm of the Sabaragamuwa University of Sri Lanka. Two hundred unsexed day-old broiler chicks of Hubbard strain were obtained from the hatchery of National Livestock Development Board, Miriswatta Farm. They were commonly brooded for 14 days in a floor brooder and fed on a commercial broiler starter feed *ad libitum* during brooding. Then the birds were

reared for another two weeks under general management (providing them the broiler diet, supply with ample water, maintaining good ventilation etc) and on the 28th day, sixty birds with similar body weight were divided into 12 groups of 5 and assigned to three treatments and a control group with 3 replicates per treatment according to complete randomized design (CRD). Four test diets were prepared by incorporating 0% (control), 0.5%, 1.0% and 1.5% curry leaves respectively to a commercial broiler finisher diet based on maize and soybean that contained all the nutrients required by broiler finishers as recommended by NRC (1994) [38].

Fresh matured leaves of *M. koenigii* plants (approximately a year old plants) were collected from natural habitats at Belihuloya area and sundried after removal of extraneous matter. The leaves were kept in oven at 50°C for 2 hrs and then ground mechanically and sieved through a fine mesh. Then the curry leaves powder was stored in airtight polythene bags at room temperature until used for the trial. Experimental diets in mash form were offered to birds *ad libitum* during 4 weeks. Birds had free access to drinking water all the time. Following 8 hr feed withdrawal period, birds were sacrifice on 42nd day by severing the jugular vein.

2.2 Collection of Gut Samples

Just after the slaughtering of poultry birds, 25 g of gut content was collected into sterile containers and immediately after collection, samples were transported to the Laboratory of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka for further investigations.

2.3 Microbiological Investigation

Samples were homogenized in sterile distilled water using a stomacher and take 1 ml of the sample for further investigation. 1 ml of gut homogenate was serially diluted in sterilized distilled water and 100 µl of each dilution was cultured on standard plate count agar (Himedia, India) plates using spread plate method in three replicates for each dilution and plates were incubated at 35± 2°C for overnight subsequently the colonies were enumerated manually.

2.4 Lipid and Glucose Profile

Blood samples were collected from three randomly selected birds from each group at slaughter. Blood was collected to vacutainers with no additives for serum separation. Blood samples were subjected to lipid profile analysis (total cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL) and Triglycerides) and blood glucose analysis by enzymatic diagnostic kits (Diasys diagnostic kits,Gmbh, Germany).

2.5 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) with $p<0.05$ considered significant (SPSS, 2000). Data were compared with one way ANOVA, followed by a Tukey's post-hoc test. For all statistical analyses, a 95% significance level was used.

3. RESULTS AND DISCUSSION

Results of the study revealed that there is a significant reduction of gut microbes in poultry which were fed curry leaves at 1% ($5.6x10^8$ CFU/g) and 1.5% ($4.5x10^8$ CFU/g) when compared to the unfed control ($8.9x10^8$ CFU/g) (Table 1). Addition of curry leaves at 1% reduced the gut microbes by 37.2% and addition of curry leaves at 1.5% reduced it by 49.1%. The study further, exhibited that there is no significant reduction of microbes with 0.5% ($8.5x10^8$ CFU/g) of curry leave addition when compared to the unfed control and the percentage reduction was 4.5 (Table 1).

Table 1. Effect of *Murraya koenigii* (curry leaves) on gut microflora of broiler chicken

% of curry leaves added to the feed	Bacterial count (CFU/g of gut content)	% reduction of gut microbes
Control* (0%)	$8,9x10^{8\ A} \pm 3.7*10^6$	-
0,5	$8,5x10^{8\ A} \pm 9*8*10^6$	4.5
1	$5,6x10^{8\ B} \pm 2.6*10^6$	37.2
1,5	$4,5x10^{8\ C} \pm 1.3*10^6$	49.1

Values in the same column with a different superscript letter are significantly different from each other (P < 0.01).
** Control feed i.e no curry leaves added, bacterial counts are expressed as Colony Forming Units/g ± Standard error*

This study in line with the literature revealed that curry leaves are effective alternative to therapeutic antibiotics [37]. Furthermore this study has reiterated and confirmed the evidence in previous research and in scientific literature, found that curry leaves possesses antimicrobial properties. Extracts of curry leaves has showed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, and steroids which could be responsible for the observed antimicrobial property [18]. Methanolic extract of curry leaves inhibited *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus uberis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Corynebacterium gravis* and *Bacillus cereus* and there was a reduction of gut microbes in the current study as well [18]. The petroleum ether, chloroform, ethyl acetate and ethanol extracts of roots of the Murraya koenigii plant were screened for phytochemical properties and antimicrobial activity for *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* and it showed that all the extracts possess remarkable antibacterial activity [39] and this study also supported the findings of our group. In accordance with this study, many other studies also had shown the antibacterial effect of *Murraya koenigii* against several microorganisms such as *Streptococcus mutans* and *Lactobacillus acidophilus* [40], *Treptococcus sanguis*, *Streptococcus salivarius* [41], *K.pneumoniae* and *P.aeruginosa* [42].

The initial body weight of birds was similar in all treatment groups. The average feed intake during the trial period varied from 117.8 to 119.3 g/head/day without a significant difference ($p<0.05$) between treatments. This observation is in agreement with previous findings that indicated that herbs, plant extracts, essential oil and/or the main components of the essential oil did not affect feed intake in broilers [43,44,45].

This study further showed that the curry leave significantly reduced the serum glucose level by 10, 13 and 16% in birds with 0.5, 1.0 and 1.5% curry leave levels respectively, when compared to the control. In a previous study researchers have found that, mahanimbine is a chemical constituent of *M. koenigii* and it was isolated from column chromatography of the petroleum ether extract of dried plant [46]. The anti-diabetic activity has been shown on the streptrozotocin induced wistar rats by using pure compound at a dose of 50 mg/kg and 100 mg/kg. The possible mechanism by which the mahanimbine decreases blood sugar level may be by potentiating of insulin effect either by increasing the pancreatic secretion of insulin from beta cells of islets of langerhans or by increasing the peripheral glucose uptake. Thus, it is possible to explain the hypoglycemic effect of curry leaves found in this study as well. The anti-diabetic components of flavonoids have been revealed in other study and have been found useful in traditional ethnic remedies. The flavonoids, hesperidin and naringin affect the expression of genes responsible for controlling the blood glucose, which will leads to lower the blood glucose level. Also it has been shown that two flavonoids also led to a decrease in the plasma and hepatic cholesterol levels and free fatty acids [47]. *Murraya koenigii* leaves induced paraoxonase 1 activity in streptozotocin induced diabetic mice decreasing blood glucose level and reducing the hyperlipidemia [48]. Similar kind of results of reducing the blood glucose levels in rats were observed by Al-Ani and his colleagues [49]. In line with the previous studies, current study also showed the blood glucose lowering effect with the inclusion of *Murraya koenigii* into the poultry diet.

As shown in Table 2, serum total cholesterol concentration was significantly lowered ($p<0.05$) in birds who received curry leave diets compared to the control group. Compared to the control, curry leave at 0.5%, 1.0% and 1.5% levels

Table 2. Effect of *Murraya koenigii* (curry leaves) on blood glucose, total cholesterol, triglycerides, HDL and LDL of broiler chicken

Curry leave level added to the feed	Glucose (dl/mg)	Total cholesterol (dl/mg)	TG (dl/mg)	HDL (dl/mg)	LDL (dl/mg)
Control (0%)	$231,7^{a} \pm 17,3$	$163.1^{c} \pm 14.1$	$40,6^{e} \pm 7,0$	$88,9^{f} \pm 9,4$	$68,5^{g} \pm 3,4$
0,50%	$209,3^{b} \pm 6,2$	$153.3^{cd} \pm 12.3$	$40,5^{e} \pm 3,5$	$104,5^{f} \pm 3,5$	$50,7^{h} \pm 8,4$
1,00%	$201,2^{b} \pm 12,2$	$142.9^{d} \pm 15.0$	$44,6^{e} \pm 6,9$	$100,5^{f} \pm 17,0$	$47,5^{h} \pm 8,3$
1,50%	$194,4^{b} \pm 7,5$	$138.8^{d} \pm 6.8$	$47,9^{e} \pm 9,8$	$106,6^{f} \pm 21,9$	$44,8^{h} \pm 7,2$

Values are expressed as dl/mg ± Standard error; values in the same column with a different superscript letter are significantly different from each other ($P < 0.05$).
**control; feed without curry leaves supplementation*

reduced the serum total cholesterol by 6%, 12.4% and 15% respectively. There was no significant difference in triglycerides and HDL levels among treatments. LDL level was significantly ($P< 0.05$) lowered by 26.0, 30.7 and 34.6% respectively in birds fed with 0.5, 1.0 and 1.5% curry leave levels. Hypocholesterolemic activity previously has been checked in aged mice, which was done by using crude ethanol extract of plant leaves of *M. Koenigii* [50]. Their findings have been confirmed by observing a decrease in cholesterol level in dose dependent manner in aged mice. The dose of 500 mg/kg was found more efficient than the 300 mg/kg and was comparable with the standard cholesterol reducing agent, Simvastatin.

Another study concluded that consumption curry leaves by the menopausal women with mild hyperlipidemia daily for 45 days as a part of lunch was effective in improving HDL and lowering total cholesterol, LDL and triacyl glycerol [51]. Aqueous and methanol leaf extract of Murraya koenigii were investigated by Vinuthan and colleagues in 2007 [52] for hypolipidemic effects on male Sprague Dawley rats suggesting that these extracts exert hypolipidemic activities in treated rats. All the birds that received curry leave diets had significantly lower ($p<0.05$) levels of LDL concentrations compared to the control group and it was lowered by 26.0, 30.7 and 34.6% respectively in birds fed with 0.5, 1.0 and 1.5% curry leave levels (Table 2). Tembhurne and Sakarkar (2010) [31] have also got the similar kind of results with rats. There was no significant difference observed in triglycerides and HDL levels among treatments. This result deviated from previous observations where increase in serum HDL level and reduction in serum triglyceride level had been shown after introducing higher doses (500 mg/kg) of crude ethanol extract of *M. Koenigii* [31]. The reason for the present observation may be the inadequate amount of compound received by the bird after ingestion of the given amount of curry leaves in the diet.

4. CONCLUSION

It was concluded that *M. Koenigii* (curry leave) posses a strong cholesterol lowering and hypoglycemic effect in broiler chicken. It reduces total cholesterol, serum LDL and serum glucose levels significantly after feeding *M. Koenigii* incorporated diets. There is a substantial antimicrobial effect in curry leaves and higher the inclusion level of curry leaves up to 1.5% dietary inclusion, the higher the antimicrobial effect.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

DISCLAIMER

I hereby declare that part of this work was previously published as a short abstract in the following conference. International conference of Agricultural Science (Aginsight 2014), Sabaragamuwa University of Sri Lanka, January 9th and 10th 2014, Pp 59.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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