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Research Article

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

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Abstract

This study was conducted to evaluate the caeca microbial population of growing grass cutters (*Thyronoyms swinderianus*) fed *Phyllantus amarus* and *Pilogstigma thonngii* leaf meal mixture as partial replacement for soya bean meal. A total of thirty five (35) weaned grass cutters of mixed sex between 5-6 weeks with an average weight of 436.1 and 437.0 grams were randomly assigned to five treatment groups in a completely randomized design (CRD). Five experimental diets designated as T1, T2, T3, T4 and T5 were formulated such that soya bean meal was partially replaced by *Piliostigma thonningii* and *Phyllantus amarus* leaf mixture (PATML). Feed and water was provided ad *libitum* throughout the experiment which lasted for 12 weeks. Data obtained was used to determine the caeca microbial population in the animal. Microbial population were influenced by the dietary treatments (P<0.05). *Escherichia coli* (*E. coli*) count in the caecum of grass cutters significantly (P<0.05) decreased in T5 compared to that of T1. However, Lactobacilli count significantly (P<0.05) increased in T2, T3, T4 and T5 compared to T1. It could be concluded that partial replacement of PATLM at 40% repopulates the caecum with beneficial bacteria, which curbs the action of pathogens and controls their population favoring eubiosis and better livestock performance.

Keywords: *Phyllantus amarus*, *Pilogstigma thonngii*, grass cutters, pathogens

Introduction

One of the important cardinals of management is feeding and it accounts for 50-70% cost of production [1] but there has recently been an increase in competition among the ingredients used in the manufacture of animal feed. There is consequently a great need for alternatives that can supply in the high demand for such ingredients, in particular for soya bean meal [2]. The use of plants (leaf meals) that are not directly used by humans as food have been suggested as one of the method to reduce feed cost and make protein available to human being [3]. Among the potential plants that are loaded with proteins, minerals vitamins and various phytochemicals are *Pilostigma thonngingii* and *Phyllantus amarus*.

Both plants are some of the numerous underexplored plants [4]. The plants are rich in phytochemicals or bioactive chemicals which enables them to perform multiple biological roles such as anti-inflammatory [5,6], antimicrobial [7], antiviral [8,9], antifungal [10], antibacterial [11], anti-inflammatory [6,12], antihelminthic [13], anticancer [14] and antidiuretic. Phytochemicals are also proposed for use as antioxidants in animal feed, which will protect animals from oxidative damage caused by free radicals [15]. The use of plants have been reported to be safe (less toxic) and scientifically proven as ideal feed additives in animal nutrition due to the varying degree of secondary metabolites [16].

Previous report has shown that combination of different plants exert synergistic effects to reduce negative consequences of enteric infections [17]. Lee [18] reported that dietary supplementation of newly hatched broiler chickens with a mixture of *Curcuma longa*, *Capsicum annuum* (pepper) and *Lentinus edodes* improved body weight gain and reduced coliform bacteria counts. Alagbe, Hassan, Phyo [19-22] reported that some plants such as thyme, lemon grass, turmeric and garlic are reliable feed additive and could provide positive advantages to the colonization as well as proliferation of lactobacilli, thereby modulating the immune system and activity of phagocytic host cells. Therefore this experiment was designed to evaluate the 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.

Materials and Methods

Site of the experiment

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Institute, Gujarat, India during the month of December to February, 2019.

Collection and processing of test materials

Fresh and mature leaves of *Piliostigma thonningii* and *Phyllantus amarus* leaves was procured from a local market in Gujarat and authenticated at biological science department of the research farm. It was air dried under shade to reduce moisture content until they were crispy to torch. The leaves thereafter were crushed separately with hammer mill to form *Piliostigma thonningii* leaf meal (PTM) and *Phyllantus amarus* leaf meal (PAM) respectively. The samples were later mixed in ratio of 1:1 to form PATML. The samples were kept in an air tight container for further analysis.

Formulation of experimental diet

The basal diet was formulated to meet the nutrient requirements of weaner grasscutters according to Adeniji [23].

Treatment 1 (Control): Basal diet + 0 % PATML

Treatment 2: Basal diet + 10.0 % PATML

Treatment 3: Basal diet + 20.0 % PATML

Treatment 4: Basal diet + 30.0 % PATML

Treatment 5: Basal diet + 40.0 % PATML

Pre-experimental operations

Prior to the commencement of the experiment the hutches were disinfected two weeks before the arrival of the animals. Cement feeders, drinkers, foot deep at the entrance of the pens were properly cleaned and kept in the store. A separate cage was also prepared to accommodate sick or culled animals.

Animal handling and management

Thirty five (35) weaned grass cutters of mixed sex between 5-6 weeks with an average weight of 436.1 and 437.0 grams were used for the experiment. The animals were randomly divided into five groups of seven grass cutter per replicate and each animal served as a replicate in a completely randomized design. Grass cutters were allowed two weeks adjustment period during which they were fed with basal diet (morning and evening), housed in an all wired cage measuring 45×53×40 cm 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 (110 – 130 g each). Fresh feed and water were provided ad libitum and all other management practices were strictly observed.

Data Collection

Performance parameters

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

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

c. Feed: gain ratio was determined from the average feed consumed by the average weight gained in each treatment.

d. Mortality rate was recorded as it occurs daily.

Caecal microbial population

At the end of the experiment (12 weeks), caeca microbial count was conducted using five (5) grasscutters per treatments, caeca contents were collected from slaughtered animal and 10-fold serial dilution method, in which of 1% peptone solution was mixed with caeca samples and poured on Mac Conkey agar plates and lactobacilli medium III agar plates, was used to determine the colony forming unit (cfu) in each gram of caeca sample by means of pour plate method. Colonies of *E. coli* and Lactobacilli were enumerated according to the method outlined by Phyo [22]. The microbial counts were determined as colony forming units (Cfu/g) of sample.

Laboratory Analysis

Proximate analysis of experimental diet was determined using methods described by AOAC [24]. Phytochemical screening of PATML was analyzed according to methods outlined by Harbone; Boham and Kocpai [25,26]. Mineral analyses were carried out using Atomic Absorption Spectrophotometer (AAS) model 12-0TA.

Statistical Analysis

All data collected was subjected to one-way analysis of variance (ANOVA) using SPSS (18.0) and significant means will be separated using Duncan multiple range tests [27] significant will be declared if $P \leq 0.05$.

Results and Discussion

Table 1 revealed the percentage composition of experimental diet basal. Diet was formulated to meet the nutrient requirements of weaner grasscutters according to Adeniji. Nutrition or feeding is one of the key cardinals of management and nutrients are known to influence the responses of animals to a disease challenge. According to Gary [28], if specific nutrients are at or below the animal's requirement, then a limited amount of nutrients will be available to meet the body's need during a time of challenge. The proximate composition of PATML is presented in Table 2. Dry matter (89.73 %), crude protein (37.21 %), crude fibre (16.22 %), ether extract (1.31 %) and ash (10.22 %). Dupe et al. (2015) reported a lower crude protein % was recorded in *Albizia odoratissima* leaf (24.43 %) and *Prosopis africana* leaf (16.20 %), similar crude fibre value of 16.20 % was recorded for *Gliricidia sepium* leaf. However, PATLM result was in accordance to the findings of Alagbe. According to Norton differences in the nutritive value of plants could be attributed to differences in species, age, parts of plants, soil type and season. Lower ether extract (1.31%) in PATLM could be as a result of low level of carotene and pigments, nonetheless, the ether extract value (PATLM) was higher than the report of Ogunbosoye and Otukoya. Ash content is a measure of the mineral content in a sample; this is a clear indication that PATLM is loaded with various minerals that are necessary for major metabolic process and physiological process in the body of animals [29].

Table 1: Chemical composition of experimental diets.

Ingredients	T1	T2	T3	T4	T5
Maize	40.00	40.00	40.00	40.00	40.00
Wheat offal	25.00	25.00	25.00	25.00	25.00
PKC	11.10	11.10	11.10	11.10	11.10
Soya meal	20.00	18.00	16.00	14.00	12.00
PATML	0.00	2.00	4.00	6.00	8.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Cal analysis (%)					
Dry matter	86.87	87.10	88.61	88.92	88.95
Crude protein	19.02	18.51	18.38	18.24	18.10
Crude fibre	7.62	7.78	7.94	8.10	8.26
Ether extract	3.89	3.78	3.67	3.56	3.46
Calcium	0.99	1.09	1.19	1.29	1.39
Phosphorus	0.41	0.47	0.47	0.58	0.63
Energy (Kcal/kg)	2754.0	2750.2	2755.0	2759.0	2761.0

*Premix supplied per kg diet :- Vit A, 10,000 I.U; Vit E, 5mg; Vit D3, 3000I.U, Vit K, 3mg; Vit B2, 5.5mg; Niacin, 25mg ; Vit B12, 16mg ; Choline chloride, 120mg ; Mn, 5.2mg ; Zn, 25mg ; Cu, 2.6g ; Folic acid, 2mg ; Fe, 5g ; Pantothenic acid, 10mg ; Biotin, 30.5g ; Antioxidant, 56mg

Table 2: Proximate composition of PATML.

Parameters (%)	*PATML
Dry matter	89.73
Crude protein	37.21
Crude fibre	16.22
Ether extract	1.31
Ash	10.22
Energy	2510.5

*Alagbe, J.O (2020)

Phytochemical analysis of PATML is presented in Table 3. The phytochemical constituents contained flavonoids (29.67 %), phenol (15.08 %), alkaloids (3.05 %), tannins (3.38 %), saponins (4.88 %), glycosides (1.33 %) and steroids (1.02 %). Flavonoid had the highest concentration while steroid had the least value. Phytochemicals are bioactive chemicals or secondary metabolites that confers plants the ability to perform multiple biological function. A lower phytochemical composition was reported in Spondias mombin leaf with saponin (4.80 %), alkaloid (2.40 %), flavonoids (2.80 %), tannin (1.47 %), oxalate (0.72 %), phytate (1.73 %) and glycosides (0.01 %). According to Windisch, bioactive chemicals in final product of plants could vary as a result of harvesting methods, plant parts used, age of plants and extraction methods. Flavonoids are known to possess both anti-inflammatory [30], antifungal [31] and antioxidant properties. Saponin have be shown to possess antimicrobial and also used for the treatment of hyperglycaemia [32]. Phenols have been suggested to possess antibacterial and antioxidant activity [33]. Alkaloid in plants function as analgesics [34] and antibacterial [35]. The main bioactive compounds of the phytochemicals are polyphenols, and their composition and concentration vary according to the plant, parts of the plant, geographical origin, harvesting season, environmental factors, storage conditions, and processing techniques [36].

Table 3: Phytochemical analysis of PATML.

Parameters	PATML	*Permissible range (%)
Flavonoids	29.67	36.11
Phenol	15.08	20.01
Alkaloids	3.05	3.50
Tannins	3.38	11.50
Saponin	4.88	7.02
Glycosides	1.33	-
Steroids	1.02	1.30

*Alagbe, J.O (2019); Alagbe (2020)

The effect of treatments on the count of microbial population in caeca of grasscutter is presented in Table 4. *E. coli count* ranged between 31.22 and 56.22 (cfu/g) and Lactobacilli count 17.22 and 35.56 (cfu/g). Except for Lactobacillus, which higher ($P<0.05$) in the PATLM treatments, Similarly, PATLM reduced ($P<0.05$) counts in the caecum when compared to the control treatment. The result obtained is in accordance with the reports of Phyo; Hassan. The gut is the primary site for digestion, fermentation, nutrient metabolism, nutrient absorption, immune regulation and development of immune tolerance. Gut microbiota may affect weight gain through regulating nutrient extraction, and modulating the immune system and metabolic signaling pathways [37].

Table 4: Effect of PATML on the caecal microbial population of grasscutters.

Parameters (Cfu/g)	T1	T2	T3	T4	T5	SEM
E.coli	56.22^a	41.19^b	38.56^c	34.40^c	31.22^c	3.47
Lactobacilli	17.22^c	21.44^b	28.70^b	29.10^b	35.56^a	1.25

SEM: Standard Error of Mean

According to Filipe, *E.coli* and other pathogenic organism are undesirable flora and are capable of causing dysbiosis especially during prolong water deprivation, stress and feed starvation leading to reduction in absorption of nutrients and eventually death in advanced cases. PATLM inclusion in the diet has also shown its ability to repopulates beneficial bacteria (lactobacilli), which curbs the action of pathogens, thus acting as a probiotic. The presence of various bioactive chemicals (phytochemicals) in PATLM allows it to perform multiple biological activities [38]. Similar observations were made by Ankri and Mirelman [39]; Lambert who reported that phytochemicals could kill pathogenic bacteria by competitive exclusion process and also due high percentage of phenolic compounds, which possess strong antibacterial properties. Phytochemicals have the ability to prevent the development of virulence structures in bacteria, such as flagella, which critical for bacterial adhesion to the gut of animals [40,41].

Conclusion

This present result suggests that PATLM is a natural growth promoter that has the ability to stabilize the GIT, increases the number of beneficial bacteria producing lactic acid and favoring eubiosis. Inclusion of PATLM repopulates lactobacilli, controls pathogenic bacteria especially during period of stress. Therefore, the partial replacement of soya bean meal with PATLM at 40 % promotes better livestock performance and does not have any deleterious effect on the health of the grass cutters.

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Conflicts of Interest

The authors declare no conflict of interest.

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