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EFFECTS OF CLOVE BUD (*Syzygium aromaticum*) AND FENUGREEK SEED (*Trigonella foenum graecum*) AS FEED ADDITIVES ON CEACAL MICROBIAL COUNTS OF GROWING RABBITS

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ABSTRACT

The use of phytogenic feed additives in livestock industry is gaining ground, there is need to evaluate its effect on ceacal microbes of rabbits when supplemented in their diets. Clove bud and fenugreek seed meals supplementation was investigated on ceacal microbial counts of rabbits. An experiment with seventy-two (72) weaner rabbits of mixed breeds and sexes weighing between 853.89-867.44 g with age range of 7 – 8 weeks were used in a 2x4 factorial arrangement (clove and fenugreek, at 0, 100, 200 and 300g levels, respectively) in a completely randomized design (CRD). The rabbits were allotted to 8 treatment groups with nine per treatment. Each treatment was replicated three times with 3 rabbits per replicate. Diet levels of 0, 100, 200 and 300g clove bud meal and 0, 100, 200 and 300g fenugreek seed meals were fed to the rabbits. Samples were collected from 3 rabbits per treatment to determine the microbial counts in the caecum. The data generated were subjected to analysis of variance (ANOVA) and means were separated using Duncan Range Multiple Test (DMRT). The results revealed significant ($P<0.05$) main and interactive effects between the dietary inclusions of clove and fenugreek in the parameters analyzed. Significant ($P<0.05$) main effects were observed in rabbits fed the feed additives (clove and fenugreek); this effect was recorded for *Escherichia coli, Staphylococcus, Coliform* and *Lactobacillus Spp. Salmonella spp* were not detected in rabbits fed the fenugreek diet while those fed the clove diets were recorded in minute counts. *Lactobacillu*s was significantly ($P<0.05$) higher and better in rabbits fed 300 g fenugreek diet (11.84×10^3cfu/g) while the control diets (5.83×10^3cfu/g and 5.80×10^3cfu/g) had the lowest counts. *Bacillus spp* was also significantly ($P<0.05$) increased, rabbits fed 300g clove (13.19×10^3cfu/g) and 200g fenugreek diets (14.85×10^3cfu/g) had higher concentrations of *Bacillus spp* compared to other treatments. This showed that as the dietary levels of clove and fenugreek increased, *Bacillus* and *Lactobacillus spp* counts significantly ($P<0.05$) increased while other microbes decreased. It was concluded that up to 300g of clove and fenugreek increased the beneficial bacteria and reduced the pathogenic ones.

Keywords: Clove bud, fenugreek seed, feed additives, rabbits, caecal counts

INTRODUCTION

Phytogenic feed additives (PFAs) are group of natural growth promoters or non- antibiotic growth promoters which are derived from herbs, spices or other plants (Zentek *et al.*, 2011). There is evidence to suggest that these PFAs have appetizing, digestion-stimulating properties, and antimicrobial effects. As a result, new commercial additives of plant origin, considered to be natural products that consumers would accept, have been proposed to livestock producers. These additives have received increased attention as possible antibiotic growth promoter replacements, they can be valuable alternatives for the health and nutrition of animals (Manan *et al*., 2012). Herbs and spices contain a

large number of antimicrobials, spasmolytic and anti- secretory compounds and raising interest in them as feed ingredients during the weaning period of animals. Resistance of entero-pathogens such as *Escherichia coli* to antibiotics occurs on rabbit farms (Cerrone *et al*., 1999), restricting the use of these drugs for prolonged periods. Therefore, feed additives with the potential to prevent digestive problems are considered as a promising alternative.

A part of rabbit farm´s profitability depends on the efficacy of weanling rabbits to grow efficiently and to rescue from morbidity and mortality during the fattening period. For this reason, natural growth promoters are commonly included in the diets of growing rabbits (Attia *et al*., 2014). Supplementation of growth promoters from different sources in rabbit diets is common and widely used to improve the utilization of nutrients (Abdel-Wareth *et al*., 2014). In addition, phytogenic compounds are not only used to control pathogenesis, but also to improve appetite, intestinal microflora, immune functions, oxidative status, growth and carcass traits when included in animal diets (DalleZotte *et al.,* 2016). Considering the affordability of these plant materials, they could be used as growth promoters in the livestock sector worldwide (Upadhyay *et al*., 2014). Most of the active phytochemicals identified in plant materials are of alkaloids, terpenes, flavonoids and glucosinolates (Alimohamadi *et al*., 2014). However, each plant has a unique combination of these phytochemicals, and therefore their biological effects are expected to be different. It is also reported that by using phytogenic flavors, mortality is reduced because of optimization of the immune system (Fortun and Boullier, 2007).

Clove (*Syzygium aromaticum*) is considered as one of the most versatile spices. They contain a large number of biological active compounds such as eugenol acetate, and beta- caryophyllene (Jimoh *et al.*, 2017), with eugenol being the most biologically active compound and makes up 70-80% of clove (Al-Shaikh and Perveen, 2017). Clove is a safe plant when consumed in concentrations lower than 1.5 g/kg (Cortés-Rojas *et al*., 2014). Many studies have evaluated the effect of clove powder on performance and immune response of broiler chickens (Mustafa, 2016). Fenugreek (*Trigonella foenum graecum*) is an herb cultivated for its seeds. The major bioactive compounds in fenugreek seeds are believed to be polyphenol compounds, such as rhaponticin and isovitexin (Khan *et al*., 2018). Being rich in phytochemical like phenols, saponin, flavonoids, alkaloids and tannins, it has been tagged as antidiabetic, anticarcinogenic, hypocholesterolemic, antioxidant and immunological booster (Wani and Kumar, 2016). Fenugreek seeds are rich in protein, fat, total carbohydrates and minerals viz. calcium, phosphorus, iron, zinc, magnesium (Gupta *et al*., 1996) and iron (Rao, 2003). Based on the medicinal value of fenugreek, it could be a possible replacement for synthetic antibiotics as a medicinal plant in monogastric diets to improve body weight gain, feed/gain ratio and reduced the cost of feed (Adil *et al*., 2015). The aim of this study is to evaluate the caecal microbial count of rabbits fed diets containing graded levels of clove and fenugreek meals as phytogenic feed additives in the diets of rabbits.

MATERIAL AND METHODS

Experimental site

The experiment was carried out at the Rabbitry Unit of the Department of Animal Science Teaching and Research Farm, Ahmadu Bello University, Zaria. Zaria is within the Northern Guinea Savanna zone of Nigeria, with Latitude 11^0 09′ 01.78″N and Longitude 7^0 39′ 14.79″E at an altitude of 671m above sea level (Ovimaps, 2021).

Sources of experimental animals and materials

The rabbits used for this study were sourced from a reputable source in Samaru, Zaria. The sunflower seed cake was sourced from Product Development Research Programme, Institute of Agricultural Research (IAR), Ahmadu Bello

University, Zaria. Clove buds, fenugreek seeds and other ingredients were sourced from Samaru market and Labar Feed Mill, Samaru, Zaria.

Processing of cloves and fenugreek and Experimental diets

The clove buds and fenugreek seeds were milled into meal and stored in airtight containers. The milled meals were later incorporated in the diets. Eight (8) experimental diets were formulated to meet the requirement of rabbits according to (Aduku and Olukosi, 1990). The experimental diets were as follows: 0, 100, 200 and 300g clove bud meal supplementation, respectively and 0, 100, 200 and 300g fenugreek seed meal supplementation, respectively as shown in Table 1.

Table 1: Gross composition of experimental diets

	Dietary levels of phytogenic feed additives (g/100kg diet)							
	Clove bud				**Fenugreek seed**			
Ingredients	**0**	**100**	**200**	**300**	**0**	**100**	**200**	**300**
Maize offal	33.50	33.50	33.50	33.50	33.50	33.50	33.50	33.50
Soybean cake	25.20	25.20	25.20	25.20	25.20	25.20	25.20	25.20
Sunflower seed cake	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80
Rice offal	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Clove meal	0	100	200	300	-	-	-	-
Fenugreek meal	-	-	-	-	0	100	200	300
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix *	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Analysis								
ME (Kcal/kg)	2677	2677	2677	2677	2677	2677	2677	2677
Crude protein (%)	16.35	16.35	16.35	16.35	16.35	16.35	16.35	16.35
Crude fiber (%)	17.82	17.82	17.82	17.82	17.82	17.82	17.82	17.82
Ether extract (%)	7.13	7.13	7.13	7.13	7.13	7.13	7.13	7.13
Feed cost (N/kg)	183.40	198.20	213.00	227.80	183.40	193.40	203.40	213.40

**Premix (grow fast) Manufactured by Animal Care Service Consult (Nig) Ltd. Lagos, Supplying the following per kg of premix: Vitamin A, 5000,00 IU; Vitamin D3 800,000IU; Vitamin E, 12,000mg; Vitamin K, 1,5000mg; Vitamin B1, 1,000mg; Vitamin B2, 2,000mg, Vitamin B6, 1,500mg; Niacin, 12,000mg; Pantothenic acid, 20.00mg; Biotin,10.00mg; Vitamin B12, 300.00mg; Folic acid, 150,000mg; Choline, 60,000mg; Manganese, 10,000mg; Iron;15,000 mg, Zinc 800.00mg; Copper 400.00mg; Iodine 80.00mg; Cobalt 40mg; Selenium 8,00 mg

Experimental design and management of rabbit

The design of 2x4 factorial arrangement (2 additives; clove and fenugreek, and 4 levels; 0,

100, 200 and 300g) in a completely randomized design (CRD) was used to conduct this study. Seventy-two weaner rabbits of mixed breeds and sexes weighing between 853.89-867.44 g and about 7 – 8 weeks old were procured. All the rabbits were randomly allotted based on average weights into 8 treatment groups with nine per treatment, each treatment is replicated thrice with 3 rabbits per replicate. They were housed in metal cages (55cm x 40cm x 40 cm size per cage) during the whole experimental period. The rabbits had access to water and feed *ad-libitum.*

Caecal microbial count of rabbits

At the end of the feeding trial, caecal contents were collected from 3 slaughtered rabbits per treatment using a sterile culture kit containing swab and sterile collection tube. The swab was placed in culture media containing nutrient and agar poured in labelled Petri dishes and placed in the collection tube with label for microbial culture. Estimation of total bacteria counts; *Escherichia coli, Coliform, Clostridium, Lactobacillus, Bacillus, Salmonella, Staphylococcus* were done using different selective media for isolation of bacteria group and characterization based on sugar fermentation using Microbact 12E kit and conventional biochemical method (OMP, 2015). This was carried out at the Department of Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) using General Linear Model (GLM) procedure of Statistical Analysis System (SAS, 2008). Significant differences among treatment means were separated using Duncan Multiple Range Test.

RESULTS

Main effects of feeding diets containing different levels of clove bud and fenugreek seed meals on caecal microbial counts of rabbits

The result of the main effect of diets containing clove bud and fenugreek seed meals on caecal microbial counts of rabbits is presented in Table 2. There were significant ($P<0.05$) main effects in *E. coli, Coliform, Staphylococcus, Lactobacillus spp.* The microbial counts for rabbits fed clove diet was higher than for those fed the fenugreek diet except for *Lactobacillus spp* which was higher for rabbits fed fenugreek diet (8.94 $x10^3$cfu/g) than for those fed clove diet (8.02$x10^3$cfu/g). Significant ($P<0.05$) differences were observed in all the parameters measured

for the inclusion level effect. It was noted that as the levels of the PFAs increased, there was decrease in *E. coli, Coliform, Staphylococcus* and *Clostridium* while *Bacillus spp and Lactobacillus spp* increased significantly ($P<0.05$). The highest count for *Bacillus spp* (13.66 $x10^3$cfu/g) was from rabbits fed 200 g inclusion level while that of *Lactobacillus spp* (10.16 $x10^3$cfu/g) was from 300 g inclusion level. It was observed that the PFAs decreased the harmful bacteria and in turn improved the beneficial bacteria (*Bacillus* and *Lactobaccillus spp).* Salmonell*a spp* was not significant ($P>0.05$) across the dietary treatments, the microbe was not detected in rabbits fed diets with supplemented additives. It was observed that as *Salmonella spp* were eliminated, there was a drastic increase of *Lactobacillus spp* in rabbits fed both diets.

Table 2: Main effects of diets containing clove bud and fenugreek seed meals and dietary inclusion levels on caecal microbial counts of rabbits

Parameters (10^3cfu/g)	Dietary PFAs			Inclusion levels (g/100kg of diet)				
	Clove	Fenugreek	SEM	0	100	200	300	SEM
Escherichia coli	7.92[a]	7.30[b]	0.07	9.24[a]	8.17[b]	7.21[c]	5.81[d]	0.10
Salmonella	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Coliforms	7.25[a]	6.30[b]	0.04	6.95[a]	6.80[b]	6.71[b]	6.62[c]	0.05
Staphylococcus	11.18[a]	8.81[b]	0.09	16.89[a]	10.46[b]	7.20[c]	5.45[d]	0.12
Clostridium	10.01	9.95	0.07	10.11[b]	11.04[a]	9.57[c]	9.20[d]	0.09
Bacillus	11.91	11.76	0.12	10.98[c]	10.53[c]	13.66[a]	12.00[b]	0.17
Lactobacillus	8.02[b]	8.94[a]	0.10	5.81[d]	8.25[c]	9.70[b]	10.16[a]	0.15

[abc]: means with different superscript on the same row differ significantly at P<0.05 SEM: standard error of mean PFA: phytogenic feed additives

Interaction effect between diets containing different levels of clove bud and fenugreek seed meals on caecal microbial counts of rabbits.

Table 3 shows the result of the interaction effect between the two feed additives (clove bud and fenugreek seed) with their inclusion levels on caecal microbial counts of rabbits. Significant (P<0.05) differences were observed in all the parameters measured with the exception of *Salmonella spp* whose presence was not detected in rabbits fed the fenugreek treatment groups. It was observed that as the inclusion level of clove increased, *E. coli*, *Coliform*, *Staphylococcus* and *Clostridium* were significantly (P<0.05) decreased. The same pattern was observed for the fenugreek diets. The result of this study showed that rabbits fed the control diets had the highest concentration of *E. coli* (8.97 x 10^3cfu/g and 9.52 x 10^3cfu/g) and *Staphylococcus* (18.30 x 10^3cfu/g and 15.50 x 10^3cfu/g), compared to those fed the different inclusion levels of the clove and fenugreek. *Lactobacillus spp* was significantly (P<0.05) higher and better in rabbits fed 300g fenugreek diet (11.84x10^3cfu/g) while the control diets (5.83 x 10^3cfu/g and 5.80 x 10^3cfu/g) had the lowest counts. *Bacillus spp* was also significantly (P<0.05) increased, rabbits fed 300g clove (13.19 x 10^3cfu/g) and 200g fenugreek diets (14.85 x 10^3cfu/g) had higher concentrations of *Bacillus spp* compared to other treatments. The least concentrations were observed from the control diets (10.10 x 10^3cfu/g and 10.09 x10^3cfu/g). This study showed that as the dietary levels of clove and fenugreek increased, *Bacillus* and *Lactobacillus spp* counts increased while other microbes decreased.

Table 3: Interaction between diets containing phytogenic feed additives (clove buds and fenugreek seeds) and inclusion levels on caecal microbial counts of rabbits

Parameters (10^3cfu/g)	Dietary levels of additives (g/100 kg of Diet)								
	Clove				Fenugreek				
	0	100	200	300	0	100	200	300	SEM
Escherichia coli	8.97^{b}	8.73^{b}	7.70^{c}	6.28^{e}	9.52^{a}	7.61^{c}	6.74^{d}	5.34^{f}	0.14
Salmonella	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01
Coliforms	7.33^{a}	7.34^{a}	7.30^{a}	7.10^{b}	6.57^{c}	6.25^{d}	6.20^{d}	6.14^{d}	0.07
Staphylococcus	18.30^{a}	11.20^{c}	9.44^{d}	5.84^{e}	15.50^{b}	9.80^{d}	4.95^{f}	5.06^{f}	0.24
Clostridium	9.61^{d}	11.72^{a}	10.30^{bc}	8.44^{f}	10.61^{b}	10.40^{bc}	8.86^{e}	9.96^{cd}	0.13
Bacillus	10.10^{e}	11.90^{c}	12.50^{bc}	13.19^{b}	10.09^{e}	10.95^{d}	14.85^{a}	10.75^{de}	0.24
Lactobacillus	5.83^{f}	7.87^{e}	8.89^{cd}	9.49^{c}	5.80^{f}	8.62^{d}	10.51^{b}	11.84^{a}	0.21

^{abc}: means with different superscript on the same row differ significantly at P<0.05 SEM: standard error of mean

DISCUSSION

According to Alagbe (2021), phytochemical substances such as flavonoids, phenols and alkaloids are capable of reducing the activities of pathogenic bacteria through competitive exclusion and promoting the proliferation of beneficial bacteria like *Lactobacillus spp*, thus playing a role of a probiotics. So, phytogenic substances have provided sufficient evidence to be safe and natural alternatives of growth promoters in animal diets to prevent microorganisms' contamination of human food and to prevent many diseases. The result of the main effects agreed with the reports of Murugesan *et al*. (2015) who reported that PFAs significantly reduced the ceacal population of *coliforms* and fortified the gut microbiota with the beneficial bacteria, such as *Lactobacillus spp*. Once the *Lactobacillus spp* are established, they might selectively exclude the pathogens from adhering due to their fast colonization, proliferation and acidification properties in the GIT (McReynolds *et al*., 2009). However, Perin *et al*. (2019) reported that diets containing tannin caused positive effects on the immune system and exerted potent bactericidal and coccidiostatic properties in broiler chickens. Fenugreek diets regulated the microbial community by increasing the number of beneficial *Lactobacillus bacteria* (8.94 x 10^3cfu/g) and eliminating the number of *Salmonella spp* (0.00 x 10^3cfu/g) drastically. The *Salmonella spp* not detected in this study is in agreement with the result of Tsavkar (2023) who fed rabbits with lime fruit juice as phytobiotic and detected no *Salmonella spp* in their ceacal samples. This finding is consistent with the previous report of Hangen *et al*. (1976) that naturally occurring *Salmonella* infections were uncommon in domestic rabbits. As observed, the higher levels of these additives have shown positive effect by increasing the population of beneficial bacteria (*Lactobacillus spp* and *Baciluus spp*) and displacing the pathogenic bacteria. The colonization of the ceacum by the beneficial bacteria suppresses the activity and habitation of potentially pathogenic species (Rinttila and Apajalahti, 2013). The results revealed that the dietary levels of clove and fenugreek do not act in bacteriocidal manner but in bacteriostatic manner, which means that it helps to colonize the ceacum with beneficial bacteria and reduces the virulence of pathogenic bacteria.

The balance of intestinal microbes helps to improve the intestinal development (Chen *et al.*, 2017). As observed in the interaction effect of this study, the number of pathogenic bacteria (*Escherichia coli, Coliform* and *Staphylococci spp*) decreased with increase in clove and fenugreek inclusion levels, the beneficial ones (*Bacilli spp and Lactobacillus spp)* were increased. *Bacilli spp* were one of the beneficial and harmless microbes in the microbiota, *Lactobacillus spp* were commensal bacteria that have long been known for their ability to activate the intestinal immune system and increase resistance to disease (Salihu *et al.*, 2020). This result agrees with the report of Murugesan *et al*. (2015) who reported that PFAs significantly reduced the cecal population of *coliforms* and fortified the gut microbiota with the beneficial bacteria, such as *Lactobacillus spp*. Once the *Lactobacillus spp* are established, they might selectively exclude the pathogens from adhering due to their fast colonization, proliferation and acidification properties of the GIT (McReynolds *et al*., 2009). *Salmonella spp* was not detected in this study. This could either be that it is not present or present in undetectable levels. This finding was consistent with the report of Tsavkar (2023) that naturally occurring *Salmonell*a infections were uncommon in domestic rabbits. According to (Singh *et al*., 2021; Alagbe, 2021), phytochemical substances such as flavonoids, phenols and alkaloids are capable of reducing the activities of pathogenic bacteria through competitive exclusion and promoting the proliferation of beneficial bacteria like *Lactobacillus spp*, thus playing a role of a probiotics.

CONCLUSION AND RECOMMENDATION

From the result of this study, it was concluded that

1. Supplementation of clove and fenugreek up to 300g/100kg diet improved the rabbit gut by increasing the beneficial bacteria and reducing the pathogenic bacteria
2. Clove and fenugreek of up to 300g have proven to be an alternative growth promoter to prevent microorganism's contamination and to prevent diseases
3. Further research is recommended above 300g supplementation

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