NITROGEN RETENTION IN WEANER RABBITS FED DIETS CONTAINING GRADED LEVELS OF CLOVE AND FENUGREEK

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ABSTRACT
The study was conducted to determine the nitrogen retention in rabbits fed graded levels of clove and fenugreek meals. Clove and fenugreek were obtained, cleaned and milled into meal as clove meal (CM) and fenugreek meal (FM). Eight (8) dietary diets were formulated; the first 4 treatments were diets containing clove meals (CM) at 0, 100, 200 and 300 g while the last 4 diets contained fenugreek meals (FM) at 0, 100, 200 and 300 g inclusion levels. Seventy-two (72) weaner rabbits having an average weight between 853.89-867.44 g were randomly assigned to 8 treatment groups with nine rabbits per treatment having 3 rabbits per replicate in a 2 x 4 factorial arrangement of completely randomized design. 100 g of feed was given on a daily basis and water provided ad-libitum The results of the nitrogen retention parameters measured were significantly (P<0.05) better in rabbits fed FM diet for the main effect. The interaction effects were significantly (P<0.05) better in rabbits fed 300 g CM diet and 200 g FM diet for nitrogen intake and nitrogen as % of intake.
Keywords: Nitrogen retention, Clove meal, Fenugreek meal, Rabbits

INTRODUCTION

Rabbits are pseudo-ruminants with the ability to convert roughages with high levels of fibre content to meat efficiently without alteration in the quality of meat produced (Iyeghe-Erakpotobor and Adeyegun, 2012). Nitrogen retention refers to the amount of nitrogen that is absorbed and retained by the body for growth and maintenance purposes, as opposed to the amount that is excreted in the urine and feces. Nitrogen retention is an important measure of the efficiency of dietary protein utilization by rabbits (Hassan *et al*., 2016). Clove is considered as one of the most versatile spices. They contain a large number of biologically active compounds such as eugenol acetate, and beta- caryophyllene. Eugenol is the most biologically active compound and makes up 70-80% of clove (Al-Shaikh and Perveen, 2017). Clove contains high concentrations of phenolic compounds such as phenolic acid, flavonoids, hydroxyphenyl propane, hydroxycinnamic acid and hydroxybenzoic acids. The content of these phenolic compounds may enhance many bioactivities of cloves ((Adefegha *et al*., 2016). Fenugreek is an herb cultivated for its seeds. Apart from the flavouring properties of its seeds, it has been able to mark its presence in ayurvedic system of medicines since ages, owing to its nutritional and therapeutic benefits. 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 phytochemicals 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 (Weerasingha and Atapattu, 2013). 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 reduce the cost of feed (Adil *et al*., 2015).

MATERIALS 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.

Sources of experimental materials, animals and experimental diets:
Feed ingredients were purchased from Labar Feed Mill and Samaru Market, Zaria while rabbits were obtained from a reputable farm in Samaru, Zaria. Eight (8) dietary treatments were formulated to meet the requirement of rabbits according to Aduku and Olukosi (1990). Maize offal, soybean meal, sunflower seed cake, rice offal, bone meal, salt, premix and clove meal (CM) /fenugreek meal (FM) was formulated as the basal diet. Diet 1: basal diet + 0 g CM, diet 2: basal diet + 100 g CM, diet 3: basal diet + 200 g CM, diet 4: basal diet + 300 g CM, diet 5: basal diet + 0 g FM, diet 6: basal diet + 100 g FM, diet 7: basal diet + 200 g FM and diet 8: basal diet + 300 g FM.

Experimental design and management of rabbit:
The design of 2 x 4 factorial arrangements in a completely randomized design was used to conduct this study. Seventy-two (72) weaner rabbits having an average weight between 853.89-867.44 g were randomly allotted to 8 treatment groups with nine rabbits per treatment having 3 rabbits per replicate. They were housed in metal cages

(55cm x 40cm x 40 cm size per cage) during the whole experimental period. Water and feed were offered to them *ad-libitum.*

Nitrogen retention studies:

At the end of the growth study, three (3) rabbits were randomly sleeted from each treatment and housed in individual metabolism crates ideal for easy collection of urine and faeces as described by Osuji *et al*. (1993). A measured quantity of the experimental diets was fed and orts were collected and weighed daily. Faeces and urine collected were also recorded daily. Urine sample were collected in a container acidified with dilute sulphuric acid (H_2SO_4) to trap the ammonia and 10% of the urine was kept in deep freezer (-20°C) until needed for nitrogen determination. The analysis was carried out at the Biochemistry Laboratory, Department of Animal Science, Ahmadu Bello University, Zaria.

Data 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 (1955).

RESULTS AND DISCUSSION

The result of the main effects of clove and fenugreek and their dietary inclusion levels on nitrogen retention of rabbits is shown in Table 1**.** FM supplementation resulted in significantly ($P<0.05$) higher nitrogen intake (2.92 g) compared to clove supplementation (2.70 g) aligns with the findings by Zeweil *et al*. (2019), who reported improved protein utilization in rabbits fed FM supplemented diets. This mechanism relates to fenugreek's bioactive compounds, particularly diosgenin and trigonelline, which have been shown to enhance intestinal absorption and protein metabolism. The improved nitrogen utilization at higher inclusion levels (200-300 g) for both additives is similar to the results reported by Ibrahim *et al*. (2018) for FM supplementation in rabbit diets, though the authors used lower inclusion levels. The optimal performance of FM at 200 g (70.70 % N retention) suggests a sweet spot for supplementation that maximizes benefits while remaining economically viable. The higher urinary nitrogen excretion with the main effect of FM supplementation (0.32 g vs 0.29 g for clove) could enhance protein metabolism as suggested by El-Shafei *et al*. (2021) and potential inefficiencies in protein utilization at certain inclusion levels.

For the inclusion levels, nitrogen intake was significantly lower ($P<0.05$) in the control group (2.57 g) compared to all supplemented groups (2.85-2.90 g), with no significant differences ($P>0.05$) among the supplemented treatments. Fecal nitrogen excretion showed significant differences ($P<0.05$) across all inclusion levels, with the highest value at 100 g (0.76 g) and lowest in the control group (0.48 g). Total nitrogen voided followed a similar pattern, with significant differences ($P<0.05$) among all inclusion levels. The variable performance of CM supplementation, particularly the lower nitrogen retention at 100-200 g, contrasts with the reports of Parapat *et al*. (2018) who reported consistent improvements in protein utilization with CM supplementation. This discrepancy might be related to the environmental factors affecting the bioactive compounds' efficacy.

The interaction effect between CM and FM and their inclusion levels on nitrogen retention of rabbits is shown in Table 2. The results revealed significant ($P<0.05$) variations in nitrogen utilization parameters

Table 1: Main effects of clove and fenugreek and inclusion levels on nitrogen retention of rabbits

	Dietary PFAs			Inclusion levels of PFAs (g/100kg diet)				
Nutrients (%)	**Clove**	**Fenugreek**	**SEM**	**0**	**100**	**200**	**300**	**SEM**
N intake (g)	2.70[b]	2.92[a]	0.10	2.57[b]	2.85[a]	2.89[a]	2.90[a]	0.13
N urine (g)	0.29[b]	0.32[a]	0.01	0.27	0.36	0.31	0.30	0.02
N faeces (g)	0.60	0.61	0.01	0.48[d]	0.76[a]	0.57[c]	0.62[b]	0.03
Total N voided (g)	0.91	0.93	0.02	0.75[d]	1.12[a]	0.88[c]	0.92[b]	0.04
N retained (g)	1.78[b]	1.99[a]	0.08	1.81[a]	1.73[b]	2.01[a]	1.99[a]	0.12
N absorbed (g)	2.08[b]	2.31[a]	0.10	2.08	2.09	2.32	2.28	0.13
N as % of intake	65.90[b]	68.10[a]	1.42	70.70[a]	60.40[b]	68.20[a]	68.50[a]	2.84

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

Table 2: Interaction between clove and fenugreek and inclusion levels on the nitrogen retention of rabbits

Parameters	**Dietary levels of additives (g/100 kg of Diet)**								**SEM**
	Clove				**Fenugreek**				
	0	**100**	**200**	**300**	**0**	**100**	**200**	**300**	
N intake (g)	2.42[b]	2.76 [ab]	2.73 [ab]	2.86 [ab]	2.71 [ab]	2.94 [ab]	3.07[a]	2.94[ab]	0.19
N urine (g)	0.25[b]	0.33[bc]	0.37[a]	0.24[b]	0.30 [ab]	0.40[a]	0.25 [b]	0.35[bc]	0.22
N faeces (g)	0.45[c]	0.78[a]	0.71[ab]	0.50[b]	0.51[b]	0.73[a]	0.43[c]	0.75[a]	0.03
Total N voided (g)	0.68[b]	1.11[a]	1.08[a]	0.73[b]	0.81[b]	1.13[a]	0.69[b]	1.10[a]	0.05
N retained (g)	1.72[b]	1.64[b]	1.64[b]	2.12 [a]	1.90 [ab]	1.82[ab]	2.38[a]	1.85[ab]	0.18
N absorbed (g)	1.97 [b]	1.98[b]	2.01[ab]	2.36 [ab]	2.20 [ab]	2.21 [ab]	2.63[a]	2.19 [ab]	0.18
N as % of intake	71.20[ab]	59.30[c]	58.70[c]	74.30[a]	70.30[ab]	61.50[b]	77.70[a]	62.70[b]	2.84

[abc]: means with different superscripts on the same row differ significantly at P<0.05 SEM: standard error of mean N: Nitrogen

measured. The nitrogen intake showed significant (P<0.05) differences, with FM at 200 g yielding the highest value (3.07 g) compared to the control group of the CM diet (2.42 g). This result agrees with the findings by Hassan *et al*. (2019), who reported improved feed intake when fenugreek was included in rabbit diets, potentially due to its appetite-stimulating properties. Urinary nitrogen excretion patterns indicated significant (P<0.05) effects, with the highest excretion observed in FM at 100 g (0.40 g) and the lowest in CM at 0g, 300 g and FM at 200 g (0.25, 0.24 and 0.25 g, respectively). This variation observed suggests a differential protein metabolism efficiency at various inclusion levels, supporting observations by El-Shafei *et al*. (2021) regarding the dose-dependent effects of phytogenic additives on protein utilization. Significantly (P<0.05) reduced fecal nitrogen excretion observed with FM supplementation at 200 g/100 kg suggests enhanced protein digestibility, possibly due to improved intestinal enzyme activity as reported by Kumar *et al*. (2020). Higher fecal nitrogen excretion observed across the treatments might indicate suboptimal protein digestion at these concentrations. Total nitrogen voided demonstrated significant (P<0.05) treatment effects, with both additives showing higher values at 100 g (1.11 g for CM and 1.13 g for FM) and 200 g CM diet (1.08 g) compared to their respective controls (0.68 g and 0.81 g). this result did not follow any pattern as it suggest that inclusion levels of CM and FM in the diets of rabbit might not optimize nitrogen retention, possibly due to insufficient concentrations of bioactive compounds to influence protein metabolism effectively. The significantly (P<0.05) improved nitrogen retention with CM at 300 g and FM at 200 g in the diet of rabbits agrees with findings by Ibrahim *et al*. (2018), who reported optimal protein utilization at similar inclusion levels. The improved nitrogen absorption observed at 200 g FM diet further supports the enhanced protein utilization efficiency. The significantly (P<0.05) higher percentage of nitrogen retained relative to intake as observed in FM at 200 g (77.70 %) and CM at 300 g (74.30 %) suggests that while both additives can enhance nitrogen retention, they require different inclusion levels for optimal performance. The lower retention rates observed with CM at 100 and 200 g (59.30 % and 58.70 %, respectively) indicate that suboptimal inclusion levels might impair protein utilization efficiency. These interaction effects demonstrate that CM and FM and their inclusion levels are crucial factors in optimizing nitrogen utilization. The superior performance of FM at 200 g/100 kg across multiple parameters suggests this combination as optimal for enhancing protein utilization in rabbit diets.

CONCLUSION

It was concluded that clove meal at 200g and fenugreek meal 300g enhanced nitrogen retention rates at 77.70 % and 74.30 %, respectively. However, fenugreek meal at 200 g/100 kg across multiple parameters suggests an optimal level for enhancing protein utilization in rabbit diets.

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