

Incorporation of *Azadirachta indica* kernel in the diet of guinea pigs: Effects on digestibility and caecal health.

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Abstract

The objective of the present study was to evaluate the effect of addition of neem (*Azadirachta indica*) kernel powder in diet on feed chemical components digestibility and on the composition of caecal microbiota of guinea pig. One hundred and thirty guinea pigs were divided equally into four groups. For 27 days, the animals were fed once daily a standard control diet (complete concentrate) or a concentrated mixture with 2.5, 5 or 7.5% (w/w) of neem kernel incorporation. The results showed that feed intake and digestibility increased significantly with the rate of kernel incorporation. In caeca content, the log number of lactic acid bacteria and *Clostridium butyricum* increased quadratically ($p < 0.001$) with the rate of kernel incorporation while that of *Escherichia coli* decreased. The results suggest that neem kernel could be used as a phytogenic supplement for guinea pigs in order to improve nutrient digestibility and microbiota quality.

Implications

Maintaining livestock intestinal health is a major challenge for breeders. Phytobiotics have shown to be promising substances for improving animal microbiota quality and thus stabilizing physiological digestion. Neem is a well known plant species used in pharmacopee. The results of this research revealed that incorporating neem kernels into guinea pigs diet led to significant improvements in nutrient digestibility, as well as marked improvement microbiota quality. These findings suggest that neem kernel in diet could be beneficial for guinea pig, promoting better nutrient absorption and improving gut health.

Introduction

Worldwide, and especially in developing countries, meat consumption continues to rise. Small-scale farming, such as guinea pig farming, can help meet the growing demand for animal protein. In Cameroon, guinea pig production is increasing, particularly among low-income families to meet basic needs for food and additional income (Imoru et al., 2019). However, feeding guinea pigs can be challenging due to their susceptibility to digestive disorders. The use of antimicrobials, whether for therapeutic, prophylactic, or metaphylactic purposes, is not recommended to address this issue, as guinea pigs are known to have a sensitivity to antibiotics in their caecal flora (Somer et al., 1955; Burgevin, 2021), not to mention concerns about antimicrobial resistance. Recent research has been conducted in Cameroon to study the effects of medicinal plants as effective alternatives to antibiotics in guinea pig farming (Djoumessi et al., 2021).

Medicinal plants contain various secondary metabolites or bioactive compounds that can promote animal health and improve performance (Windisch et al., 2008). These compounds can directly act on pathogenic bacteria as antimicrobials (Abd El-Ghany et al., 2014) or hinder the adherence of pathogenic bacteria to the intestinal mucosa by blocking certain membrane receptors (Windisch et al., 2008). They can also act as prebiotics, providing specific substrates and stimulating the growth of beneficial bacteria, or function as growth promoters (Allen et al., 2013; Abd-Elaziz et al., 2023). Interestingly, plants

can modulate the microbiota-intestinal-immune system axis through their extensive antioxidant and anti-inflammatory properties (Gheisar et al., 2018). In many species, plants increase the activity of digestive enzymes, thereby improving feed conversion and production parameters (Gheisar et al., 2018; Soltan et al., 2023). Improvements in digestive function also have been linked to the growth of beneficial bacteria – especially lactic acid bacteria such as lactobacilli and bifidobacteria – in the caeca of broiler chickens supplemented with phytobiotics, (Attia et al., 2017; Sowmiya et al., 2023). These bacterial groups enhance host health by interacting with and training the immune system, allowing the host to allocate resources to production traits (Al-Yassiry et al., 2017; Nath et al., 2023). Garlic, turmeric, and neem are among the plants that proved positive effects on such parameters. Neem kernels (*Azadirachta indica*) produce a wide variety of compounds (flavonoids, terpenoids, lignins, sulfides, polyphenols, carotenoids, coumarins, saponins, and sterols) with antimicrobial activity (Tchinda et al., 2021; Wylie et al., 2022). These compounds have shown promising results in improving the health and production parameters of various animals, such as broilers (Mafouo et al., 2019) and rabbits (Mohammed et al., 2021). They enhance energy-related intestinal functions and blood metabolites, contributing to animal health and productivity (Rehman et al., 2023). They also stimulate bile production and promote its secretion in the intestine, facilitating emulsification, waste elimination, toxin removal, and nutrient absorption (Odoh and Bratte, 2015), thereby contributing to animal health and productivity. In poultry, the addition of neem oil to feed rations has significant modulating effects on growth, intestinal ecosystem, and immune responses (Shihab et al., 2017). To our knowledge, the effects of neem kernel supplementation on guinea pigs have not yet been reported in the literature. This study aims to investigate the impact of neem kernel intake in the diet of guinea pigs on nutrient digestibility and the concentration of certain bacteria in the caecal content.

Materials and method

Geoclimatic characteristics of the study area

The study took place from August to September 2022 at the Application and Research Farm (FAR) of the Faculty of Agronomy and Agricultural Sciences (FASA) of the University of Dschang. Dschang is located at 05°26 latitude North, 10°26 longitude East, and culminates at an average elevation of 1420 m in the agroecological zone of the highlands of western Cameroon.

Plant material (*Azadirachta indica* kernel) and its origin

Neem seeds were harvested from the locality of Garoua (tropical type climate, mean temperature 25.4°C; mean rainfall 1005 mm) in the Northern Cameroon region. They were then transported to Dschang, where they were peeled. The kernels obtained were naturally dried at a temperature of 25 to 30°C to remove residual moisture and grounded using a local mill with a 1 mm diameter mesh. One kilogram of kernel was preserved in hermetically sealed plastic bags to prevent possible contamination and oxidation and later transported to the Laboratory of Foodstuffs and Animal Nutrition of the University of

Liege for chemical analyses. The chemical composition of neem kernels was as follows as % DM: crude protein 27.3, ether extract 47.7, crude fiber 18, ash 4.7.

Animal and their management

One hundred and thirty (130) English-bred guinea pigs, 450 ± 50 g average initial weight, 3 to 4-months-old, were purchased from breeders in the locality of Dschang. All animals were fed feedstuffs (*Trypsacum laxum* hay) for 7 days to standardize the digestible functions. After that, forty animals were used for the evaluation of nutrient digestibility and 90 for the caecal microbiota characterization. Vitamin C was fed daily in drinking water at a rate of 240 mg per liter during the entire trial period.

Feed formulation

The ingredients of the total mixed rations were purchased from an approved supplier of livestock ingredients. Four rations were formulated with increasing levels of neem kernel powder (0, 2.5, 5 and 7.5% of the diet). After formulated, the feed rations were granulated and a 100g sample of each one was taken for further analysis. The proportions of the different ingredients used, and the nutritional value of the diets are reported in Table 1. The ration composition took into account the nutritional needs for guinea pigs (NRC, 2006).

Evaluation of in vivo ingestion and digestibility

The forty animals were divided into 4 treatment-groups of comparable weights and housed in individual cages according to a completely randomized bloc design with 10 replicates per treatment. Then they were adapted for 10 days to the experimental rations, during which, the quantities of feed served were gradually adjusted to the animal consumption. The experimental period of digestibility measurement lasted 10 days; 40g of each of the rations was served each morning (8:00 am), after refusals and faeces had been collected and weighed. Feed intake was calculated as follows:

Fed intake (g) = Amount of feed served – Amount not consumed.

The apparent digestibility coefficients of Dry Matter (aDC DM), Organic Matter (aDC OM), Crude Fiber (aDC CF), Crude Protein (aDC CP), Extract Ether (aDC EE), Acid Detergent Fibre (aDC ADF) and Neutral Detergent Fiber (aDC NDF) were calculated using the formulas proposed by Roberge and Toutain (1999):

$$\text{aDC fraction (\%)} = \frac{\text{Ingested fraction} - \text{Faecal fraction}}{\text{Ingested fraction}} \times 100$$

Characterization of selective caecal microbiota

Following the adaptation of guinea pig to hay (day 0), out of the 90 animals in the second group 10 were randomly selected and sacrificed to determine the initial composition of the caecal microbiota. After a 10 days adaptation period similar to that of the digestibility group, forty animals (10 per group) were sacrificed, and the remaining forty on day 20 of the experiment.

The animals were slaughtered by cervical dislocation and then eviscerated. After each sacrifice, the caecum of the animal was sectioned, and homogeneous samples contents were taken with swabs. The swabs were aseptically stored in sterile boxes in the refrigerator from the FASA Physiology and Animal Health laboratory at -4°C for bacteria quantification of *Escherichia coli*, Lactic acid bacteria, and *Clostridium butyricum* spp. These bacteria were identified on the following selective and specific cultures: MRS agar, Mac Conkey agar and Reinforced Clostridial Medium (RCA) agar following the method described by Benson et al. (2002).

At the laboratory level, 0.01 g of fecal sample from each animal was collected and placed in Eppendorf tubes filled with 990 µL of phosphate 1 buffered saline solution and 8 dilutions were prepared. The samples were placed on agar plates. After incubation, the bacterial colonies were counted according to Langhout et al. (1999). The counted bacteria were expressed as log CFU g⁻¹ of caecal digesta. *Escherichia coli* were counted on MacConkey dark pink agar plates after 24 hours of aerobic incubation at 37°C. *Clostridium butyricum* were counted on a Reinforced Clostridial Medium (Merck, Germany), yellowish brown after anaerobic incubation at 37°C for 24 hours (Hirsch et Grinsted, 1954). Lactic acid bacteria were counted on a light brown agar of Man, Rogosa, and Sharpe (Merck, Germany) after anaerobic incubation at 37°C for 48 hours (Argyri et al. 2013).

Chemical analysis

The feed and fecal samples were pre-dried by drying in oven at 60°C to constant weight and then milled separately through a 1 mm sieve before analysis. Analytical Dry matter was determined according to Method 934.01 from AOAC. Diets and feces were analyzed for crude protein content (CP, Method 954.01 AOAC, 1990), Ether extract (EE) with petroleum ether as solvent (Method 920.39, AOAC, 1990), Ash (Method 942.05, AOAC, 1990), Crude fiber (method 962.09), ADF and NDF with Ankom Technology F57 fiber filter bags (method 973.18, AOAC, 1990). Phosphorus (P; Method 965.17, AOAC 1990) was obtained using a UV-vis spectrophotometer. Calcium (Ca) was determined by titration with a standardized solution of ethylenediaminetetraacetic (EDTA) as described previously (Hunt 1963). Non-nitrogen extract was calculated by difference.

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) on the Statistical Analysis System software (version 9.4, SAS Institute, Cary, NC, USA), using a general linear model (GLM). Each animal was used as an experimental unit. Before statistical analysis, fecal microbiota concentrations were log-transformed. The main effects of neem almond powder, dose and their interaction with time were tested.

The statistical model was :

$$Y_{ijk} = \mu + \alpha_i + e_{ik}$$

: Y_{ijk} = Performance i on animal j having received treatment k;

μ = Overall Mean;

α_i = effect of the rate of incorporation of neem kernels powder i ;

e_{ik} = residual error.

All dependent variables were tested for normality using the Univariate procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4). Orthogonal polynomials were performed to determine linear and quadratic effects of increasing level of neem kernels in diets. Data from guinea pigs fed diets containing kernels were compared with that from guinea pigs fed control diet using orthogonal contrasts. Lsmeans differences were evaluated using PDIFF option and all results were reported as LSMEANS followed by SEM. When significant, quadratic equations were derived on kernel incorporation level to estimate optima value. Significance levels were defined at $P < 0.05$.

Results

Feed intake

The data presented in Table 2 show that the guinea pigs fed the rations containing neem kernel had a higher DM and nutrient feed intake ($P < 0.001$) when compared to the control animals. Nutrient intake both increased linearly and quadratically with the level of kernel incorporation ($p < 0.001$). The CF and ADF intake especially were the highest in the groups R_5 and $R_{7.5\%}$. The optimum incorporation of neem kernel powder to maximize dry matter feed intake was found to be 5.84%.

Table 2
Apparent feed intake of guinea pigs fed rations containing different levels of neem kernel powders.

	¹ Dietary Rations				SEM	P > F		
(%DM)	R ₀	R _{2.5%}	R _{5%}	R _{7.5%}		Model	Linear	Quadratic
n	10	10	10	10				
DM	25.44 ^a	30.84 ^b	31.11 ^b	31.31 ^b	0.57	0.001	0.001	0.004
OM	19.13 ^a	23.20 ^b	24.07 ^b	24.30 ^b	0.45	0.001	0.001	0.001
CF	1.93 ^a	2.53 ^d	2.24 ^c	2.03 ^b	0.41	0.001	0.056	0.001
CP	4.35 ^a	5.81 ^b	6.37 ^c	6.54 ^c	0.11	0.001	0.001	0.001
EE	0.73 ^a	0.92 ^b	0.97 ^{bc}	1.00 ^c	0.01	0.001	0.001	0.001
ADF	4.14 ^a	5.30 ^b	5.60 ^b	5.70 ^b	0.10	0.001	0.001	0.001
NDF	9.06 ^a	12.71 ^b	13.24 ^c	13.38 ^c	0.24	0.001	0.001	0.001
NNE	11.39 ^a	13.43 ^b	13.39 ^b	13.89 ^b	0.57	0.001	0.013	0.205

¹ dietary treatments: R₀– control diet, R_{2.5}, R₅, R_{7.5} – diets mixed with 2.5, 5, and 7.5% of neem kernel, respectively; n = 10: number of animals per treatment, SEM – standard error of the mean. ^{a, b, c} means with different superscripts in the same row are significantly different at P < 0.05. DM = Dry matter; OM = Organic matter; CF = Crude fiber ; CP = Crude protein; EE = Ether extract; ADF = Acid Detergent Fiber ; NDF = Neutral Detergent Fiber.

Digestibility

The incorporation of neem kernel significantly (P < 0.001) increased the digestibility all of the chemical components excepted for OM (Table 3). Digestibility parameters values increased linearly and quadratically with the level of neem incorporation, but the three experimental groups did not differ from each other. Considering the significativity of the quadratic contrast, the optimum incorporation of neem kernel powder to improve dry matter digestibility was found to be 5.43%.

Table 3
Apparent digestibility of guinea pigs fed rations containing different levels of neem kernel powders.

¹ Dietary treatments					SEM	P-value		
(%)	R ₀	R _{2.5%}	R _{5%}	R _{7.5%}		P > F	Linear	Quadratic
n	10	10	10	10				
DM	71.84 ^a	77.24 ^b	77.82 ^b	77.76 ^b	0.78	0.001	0.001	0.008
OM	76.28	77.21	77.03	77.15	0.74	0.836	0.438	0.678
CF	44.60 ^a	61.51 ^c	52.53 ^{ba}	47.89 ^a	2.71	0.001	0.001	0.001
CP	75.24 ^a	84.27 ^b	85.54 ^b	85.76 ^b	0.56	0.001	0.001	0.001
EE	39.39 ^a	65.00 ^b	73.66 ^b	70.77 ^b	3.25	0.001	0.001	0.001
ADF	62.26 ^a	68.58 ^b	68.69 ^b	69.58 ^b	1.19	0.001	0.002	0.028
NDF	72.38 ^a	76.87 ^b	76.31 ^b	76.25 ^b	0.81	0.001	0.004	0.008
NNE	79.27 ^a	73.90 ^b	73.13 ^b	73.13 ^b	1.05	0.001	0.000	0.008

¹ dietary treatments: R₀– control diet, R_{2.5}, R₅, R_{7.5} – diets mixed with 2.5, 5, and 7.5% of neem kernel, respectively; n = 10: number of animals per treatment, SEM – standard error of the mean. ^{a, b} means with different superscripts in the same row are significantly different at P < 0.05. DM = Dry matter; OM = Organic matter; CF = Crude fiber ; CP = Crude protein; EE = Ether extract; ADF = Acid Detergent Fiber ; NDF = Neutral Detergent Fiber.

Caecal microbiota

Escherichia coli

The level of *E. coli* was significantly influenced (p < 0.001) by the time and level of incorporation of neem kernel in the rations compared to the initial time and control ration (Fig. 1). Guinea pigs fed rations containing 2.5, 5, and 7.5% kernel had lower levels of *E. coli* (p < 0.001), compared to the initial time (day 0) and to the control group. Guinea pigs that received the experimental diets had comparable values (p > 0.05) between them. On the other hand, the effect of the neem kernel was more pronounced at d20 of the experiment (group x time interaction effect significant at P < 0.001).

Lactic acid bacteria

Both the level of incorporation of *Azadirachta indica* kernel in diet and time significantly influenced (p < 0.05) the population of lactic acid bacteria in the caecum of guinea pigs compared to the initial time (day

0) and the control ration (Fig. 2). Initially before group allocation, the levels of were significantly lower when compared to d10 and d20. They increased with time and quadratically with the level of incorporation of neem in the rations. A maximum effect was observed at 2.5% incorporation regardless of the time considered.

Clostridium butyricum

The level of incorporation of *Azadirachta indica* kernel in the diet as well as the application time significantly influenced ($p < 0.05$) the population of *Clostridium butyricum* in the cecum of guinea pigs compared to the initial time (day 0) and the control ration (Fig. 3). Initially before group allocation, the levels of *Clostridium butyricum* was significantly lower when compared to d10 and d20. The level of bacterium increased with time and quadratically with the level of incorporation of neem in the rations, a maximum effect being observed at 2.5% incorporation regardless of the time considered.

Discussion

The present experiment aimed to evaluate the effect of dietary addition of neem (*Azadirachta indica*) kernel powder on nutrient digestibility and on composition of selected microbial populations of the caecal microbiota of guinea pig. Only one mortality case was reported in the control group and none in the experimental groups. Additionally, guinea pigs fed different rations showed no clinical signs of morbidity. In the context of this experiment, neem kernel thus could be considered as safe for the animals.

Food intake

New techniques are urgently needed to combat the digestive disorders that are inherent to the imbalance of the caeca flora in the guinea pig. The antimicrobial properties of neem as leaves or as dietary supplement have been reported by Tchinda et al. (2021) and Wylie et al. (2022), to improve productivity and health of laboratory animals (mice, rats, guinea pigs and rabbits). Consequently, in the present study, neem kernels have been used as a prophylactic method to alleviate this issue. The supplementation of neem kernel had positive effects on feed intake, highlighting the palatability of the product in the rations. Moreover, digestibility and microbial flora were improved.

Feed intake in the experimental groups was higher than in the control one. Contrary to expectations, the bitter taste (Maji et al. 2021) of the almonds did not have negative effect on feed intake of the animals. Like other plants, neem contains anti-nutritional factors (saponins, azadirachtin), which could induce a decrease in feed consumption if tolerable doses are exceeded (Adjorlolo et al., 2016). Through the findings of this study, it can be established that the incorporation of 2.5 to 7.5% of neem kernel in feed increases the dietary intake of guinea pigs. These results are not in agreement with the work of El-Bolkiny et al. (2022), who showed that the addition of 50 mg/kg of neem leaf extract reduced the dietary intake in rabbits. But the results confirm those of El-Zaiat et al. (2022), and Jack et al. (2020), who showed that the addition of 35mg/kg of leaf powder in rams and 5% neem fruit in sheep diets was safe

and acceptable, with positive effects on their daily intake. However, the maximum effect level of 5.84% on DM feed intake calculated in the context of this experiment must be taken with cautious and probably would not reflect the reality.

Digestibility

Neem kernel significantly improved the digestibility of DM of most feed components excepted OM and NNE, the digestibility of which numerically decreased. The lower digestibility of OM thus may be explained by that of NNE. This suggests that, whatever the level of incorporation, neem kernel induced a negative effect either on digestibility of starch or soluble sugar, or decreased the level of soluble fibers fermentation. In the first case, some endogenous enzymatic inhibition could occurred, and in the second hypothesis, some soluble-fiber fermentative population of microbiota may have been impacted. The mode of action of neem in animal feed is known to alter the microbiome ecosystem (Maffo et al. 2019; Chachar et al., 2022; Rehman et al., 2023). Based on our data, the potential responses of neem kernel may refer to a different selective mechanism, which may have resulted in differences in nutrient intake, fermentation level, and digestion efficiency when compared to the control group. The antimicrobial mechanism of phytogetic supplements can be seen as some growth inhibition of Gram-negative microflora and promotion of Gram-positive microbial proliferation (Chachar et al., 2022). In this experiment, the addition of neem almonds to feed rations have promoted the number of two Gram-positive species or families bacteria (lactic acid bacteria and *C. butyricum*) and decreased that of one Gram-negative species (*E. coli*). Cobellis et al. (2016) observed results in the same vein in rumen with neem essential oil. Neem kernels indirectly could have contributed to stimulate nutrient digestibility, and especially cellulose digestibility, when compared to the control group.

It could be noticed that the incorporation of neem kernel in the diet of guinea pigs resulted in a significant increase in the digestibility of DM, CF, CP, EE, ADF, and NDF compared to the control group. These results agree with Jack (2018), reported by Jack et al (2020), who showed that the addition of neem fruit in the ram's diet, increased the digestibility of hemicellulose. The improvement in nutrient digestibility could be attributed to the phenolics compounds present in the kernels. These compounds stimulate the morphology and activity of the digestive system and improve nutrients absorption (Kamboh and Zhu, 2014; Malik et al., 2020). In addition, phenolic compounds in neem kernel can alter the cell membrane of pathogenic bacteria, inhibiting their growth and survival, while leaving beneficial bacteria relatively intact. This will improve gut physiology and immune response (Mosele et al. 2015; Lima, et al., 2019). Anthocyanidin in almonds have been reported to increase the growth of potentially beneficial gut bacteria, the activity of endogenous digestive enzymes in the small intestine, and the digestibility and absorption of nutrients (Ketnawa et al., 2022), likely due to change in the structure of the microflora.

The increase in CP digestibility could be a result of the protein binding ability of the phytochemicals of neem kernel formed with dietary CP (Patra et al., 2012) but in the case of non-ruminant animals, this hypothesis is hard to support because protein is not degraded in a forestomach. Researchers have shown that the inclusion of various plant extracts improves digestion and absorption of nutrients in the

intestine. Some authors argued that the inclusion of plant extracts in the diet of chickens leads to an increase in the secretion of intestinal mucus (Tsirtsikos et al. 2012), thus increasing the digestibility of nutrients through the action of digestive enzymes that help break down nutrients in the digestive tract, especially carbohydrates and proteins. This facilitates digestion and absorption of nutrients (Oluwafemi et al., 2020).

Finally, the optimum level of DM digestibility (5.43%) is compatible with that of DM intake (5.84%).

Microbiota

The use of colony counting of caecal microbiota in this study was chosen for practical and cost reasons. The method is cheaper than advanced ones such as high-throughput sequencing or quantitative PCR. It can be performed with standard laboratory equipment and requires fewer financial resources. This method allows a direct and quantitative measurement of the number of bacterial colonies present in a sample, which can be particularly useful for quantifying bacterial load or evaluating changes in microbiota composition. Finally, the method it is a well-established and standardized in many laboratories, making it easier to compare results between different studies and laboratories (Benson, 2002). Colony counting can reveal significant changes in the composition of the caecal microbiota in response to specific nutritional interventions or treatments. For example, changes in the number of colonies of certain bacterial species may indicate a microbiota response to a particular diet or bioactive agents. This is useful for identifying changes in bacterial composition in response to dietary or therapeutic interventions.

Analysis of the caecal microbiota allows to explore animal health state (Odoh and Bratte, 2015). The microbiota helps to digest poor-quality food, improving host animal use of nutrients and modulating the development and function of the digestive and immune system (Rehman et al., 2023). According to Reda et al. (2020), establishing a state in favor to beneficial microorganisms and detrimental to harmful ones is a crucial factor for improving animal health. In this experiment, neem almond powder, with its bioactive compounds (azadirachtin, phenols, flavonoids, etc.), could have modulated positively gastrointestinal microbial composition and consequently guinea pigs health. These compounds act by forming complexes with certain proteins of the bacterial membrane of pathogens, thus inactivating their enzymes. These disturbances result in the release of cellular content and possibly the death of the microorganism. (Serrano et al., 2009). However, a more comprehensive understanding of the phenomena would require 16s-DNA analysis of microbiota.

The current study showed that neem kernel supplementation strongly affected the load of some microbial gut. Up to 7.5% of neem kernel in diet decreased in a linear way the concentration levels of pathogenic *E. coli* in caeca content of guinea pigs while they had the opposite effect on families or species of favorable bacteria (lactic acid bacteria and *C. butyricum*). The effect also increased with time. This could be associated with the improved growth performance of the guinea pigs. Neem antimicrobial activity may be related to the presence of triterpenoids, phenolic compounds, carotenoids, steroids, flavonoids and azidarachtin (Odoh and Bratte 2015). This reduction in bacterial load is in line with the

work of Rehman et al. (2023), who found that neem antibacterial properties reduced the rate of pathogenic bacteria in broilers. Additionally, Chachar et al. (2022) noticed that the use of phytobiotics decreased the number of *E. coli* in the gastrointestinal tract of compared to the control group. Odoh and Bratte (2015), observed a reduction in enterobacteria in the feces of laying hens while evaluating the feed inclusion of several levels of neem dry leaves. They also observed that a 10% feed inclusion of such leaves could be used as antimicrobial substance and natural growth promoter in diets. These results corroborate previous studies that also demonstrated the efficacy of neem leaf extracts against *E. coli* (Singh et al., 2023).

Escherichia coli is a pathogenic bacteria capable of causing disease in animals. The lower number of pathogenic bacteria recorded in R_{2.5}, R₅, and R_{7.5}% rations could indicate that neem kernel had an antimicrobial effect, possibly due to the presence of phytochemicals (triterpenoids, phenolic), which can prevent dysbiosis and preserve gut flora balance (Chachar et al., 2023). Phenolic and terpene compounds characterize neem kernels, and are at the origin of their antimicrobial activity. The latter are generally described as a weakening of the cytoplasmic membrane of microorganisms (Burt, 2004). These lead to an increase in permeability that impairs cellular activities such as energy production, membrane transport or metabolic functions. These disturbances lead to a release of the cellular content and possibly the death of the microorganism.

The number of *Lactobacilli spp.* and *C. butyricum* increased significantly with time and almond concentration. *Lactobacillus spp.* and *C. butyricum* are beneficial bacteria that produce organic acids and can modulate the immune response and improve the animal resilience, thereby promoting a healthy gut (Wu et al., 2023). The overwhelming conclusion of the majority of these studies is that the phytochemicals of *A. indica* have antimicrobial activities against several pathogens while promoting the multiplication of beneficial bacteria (Ibrahim and Kebede, 2020). A healthy gut is a more efficient digestive organ, able to mount an adequate defense against disease and easily cope with nutritional and environmental alterations.

Limitation of study

The intermediate diets (R2.5 and R5) were fully formulated and not resulted from mixing from R0 and R7.5. This could have led to possible bias in the results observed. Moreover, the different diets were not fully iso-nitrogenous. To some extent, this may have led to partial collinearity.

Conclusion

Up to 7.5% in the diet, neem kernel showed no harmful for fattening guinea pigs and had positive effects on dietary intake and digestibility of several chemical components. Moreover, strong positive effects have been observed on caeca microbiota. This suggests that neem kernel could be a useful ingredient in the diet of guinea pigs.

Declarations

Data declaration

The data will be available from the reviewers upon request.

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Author contributions

Djournessi Tobou France Gina generated the research idea, designed the study, designed and conducted experimental trial and analysis, interpreted the results, and wrote the manuscript. Hornick Jean Luc participated in the design of the study, analysis, and reading of the manuscript. Tendonkeng Fernand participated in the design of the study and reading of the manuscript. Tchiegang Clerge, Mezajoug Kenfack Blandine Laurette, Miegoué Emile and Mubé Kuietché Hervé participated in the overall design process. Fokom Wauffo David and Zambou Dongmo Delmas Kesnel participated in the design of the survey form and in conducting the data collection.

Ethical approval

This study was carried out in strict accordance with the recommendations of the ethical approval guide of the Council for Control of Animal Experimentation. The protocol was approved by the Ethics Committee on Animal Experiments of the University of Dschang, Cameroun (license number: 314/14/182/Uds/FASA/DZOO).

Ethics statement

Applicable.

Ethical approval

Applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Conflict of interest

The authors declare no competing interests.

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Table 1

Table 1 is available in the Supplementary Files section.

Figures

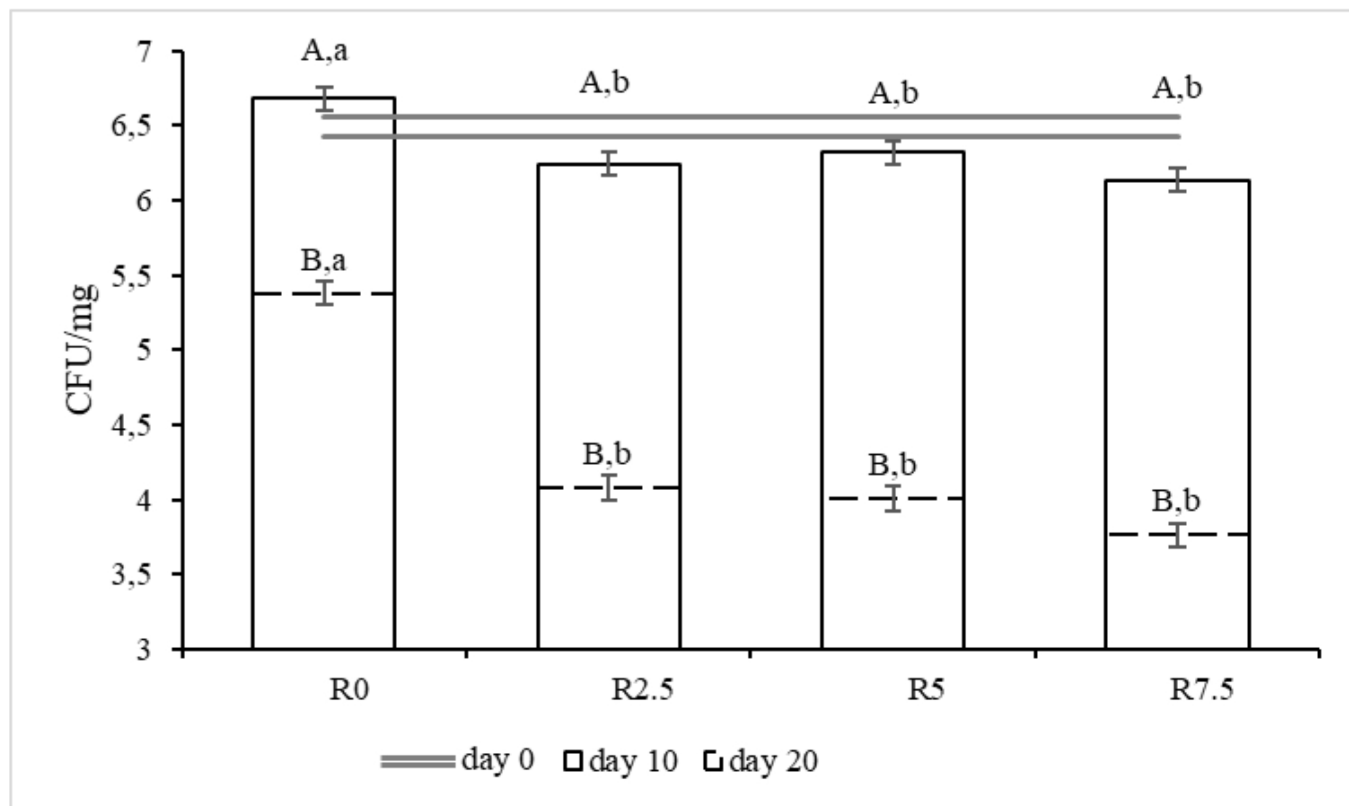


Figure 1

Concentration of *Escherichia coli* at different time in caeca content of guinea pigs fed rations containing different levels of neem kernel powders.

R₀: control ration; R_{2.5}, R₅, R_{7.5}% - rations containing 2.5, 5 and 7.5% neem seed respectively; Number of animals per treatment = 10. ^{a, b} – within time lsmeans are significantly different at P < 0.05. ^{A, B} – within group lsmeans are significantly different at P < 0.05. The two parallel lines show the confidence interval of the mean observed at the end of the transition period.

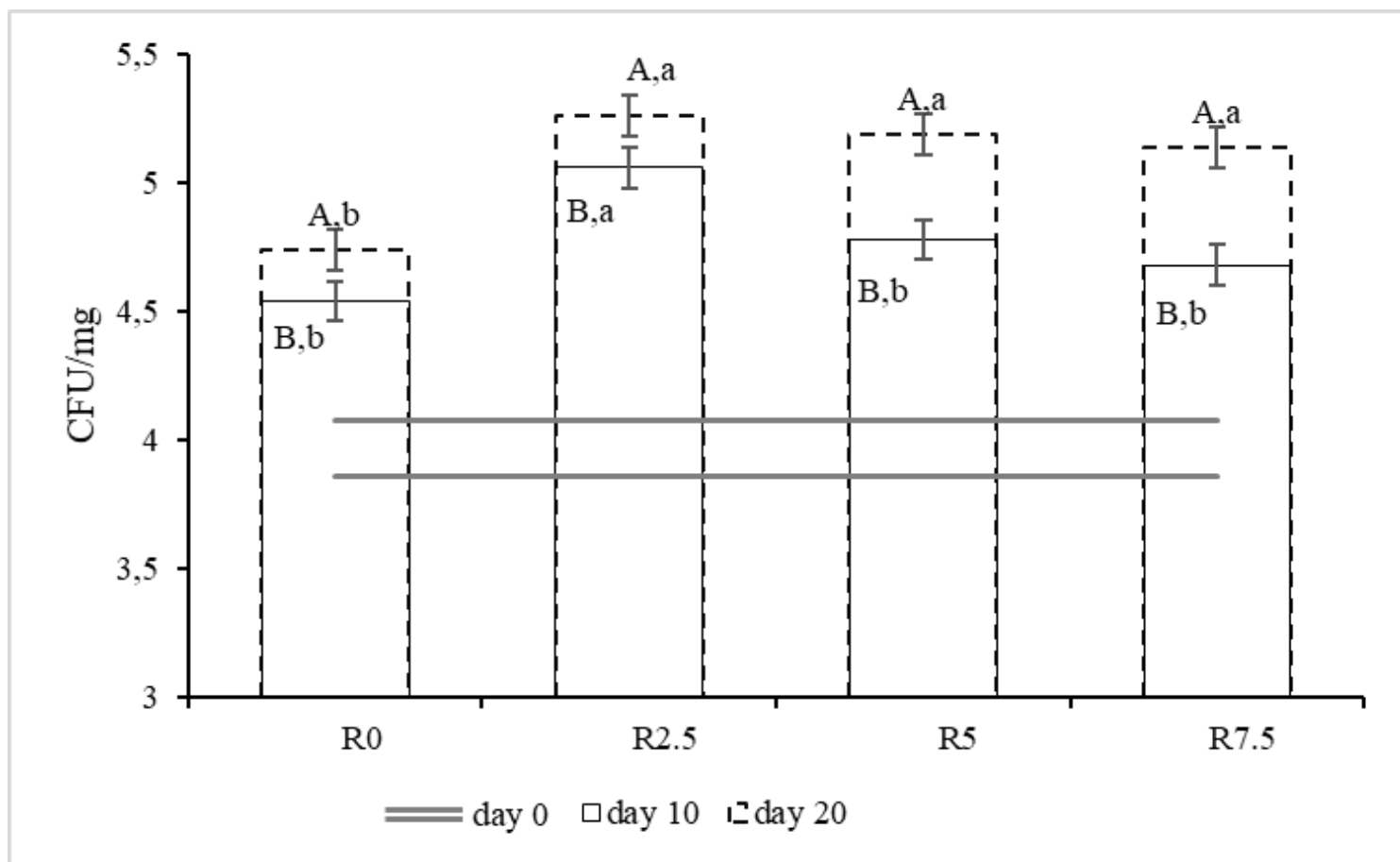


Figure 2

Concentration of Lactic acid bacteria at different time in caeca content of guinea pigs feed rations containing different levels of neem kernel powders.

R₀: control ration; R_{2.5}, R₅, R_{7.5}% - rations containing 2.5, 5 and 7.5% neem seed respectively; Number of animals per treatment = 10. ^{a, b} – within time group-Ismeans are significantly different at $P < 0.05$. ^{A, B} – within group time-Ismeans are significantly different at $P < 0.05$. The two parallel lines show the confidence interval of the mean observed at the end of the transition period.

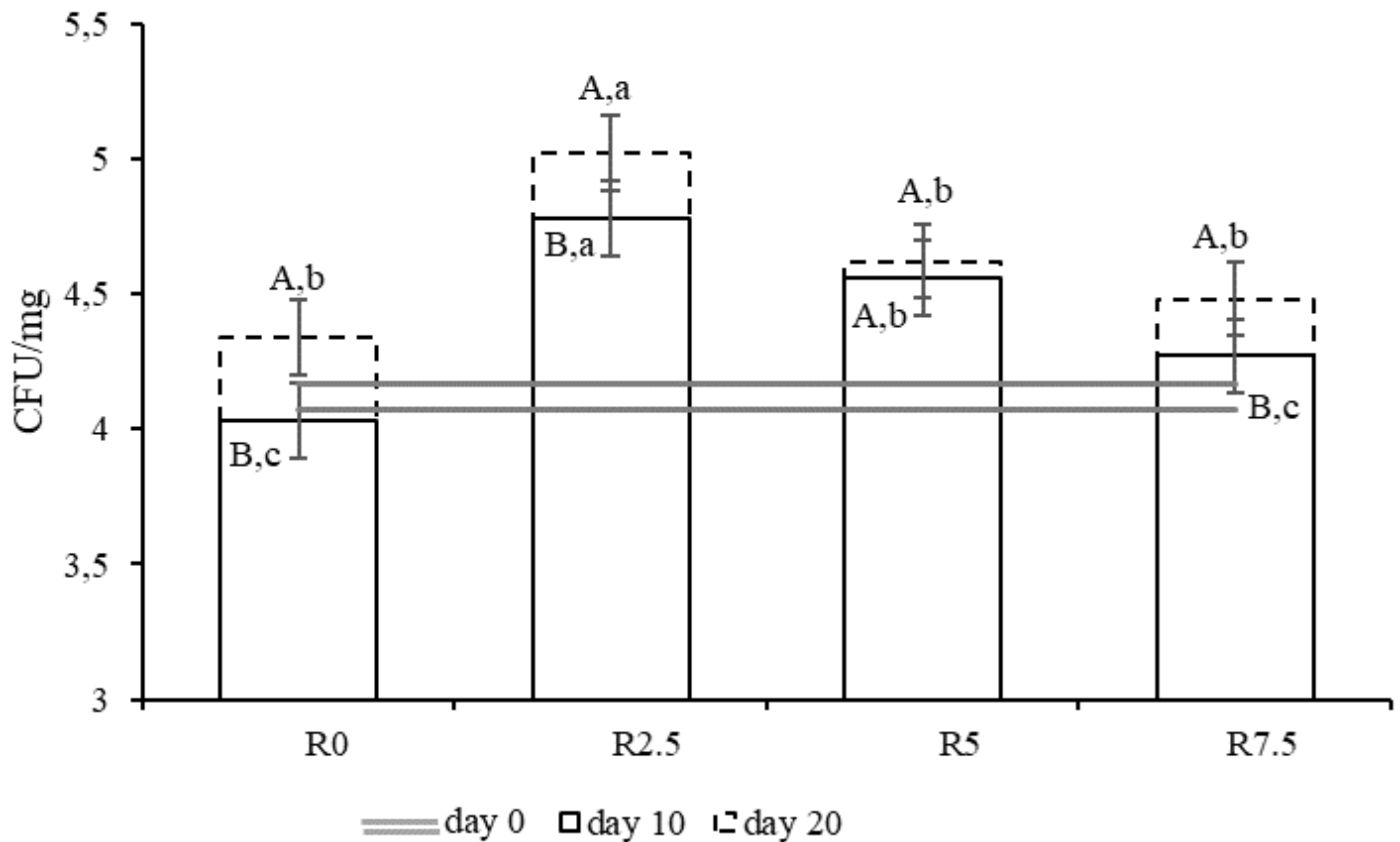


Figure 3

Concentration of *Clostridium butyricum* at different times in caeca content of guinea pigs' feed rations containing different levels of neem kernel powders.

R₀: control ration; R_{2.5}, R₅, R_{7.5}% - rations containing 2.5, 5 and 7.5% neem seed respectively; Number of animals per treatment = 10. ^{a, b} – within time group-Ismeans are significantly different at P < 0.05. ^{A, B} – within group time-Ismeans are significantly different at P < 0.05. The two parallel lines show the confidence interval of the mean observed at the end of the transition period.

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