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Addition of neem seeds (*Azadirachta indica* A. Juss) to fattening pig diets

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Abstract

Neem products are biologically and pharmacologically active and could therefore serve as alternatives to antibiotics in pig feed. This study evaluated the effects of adding ground neem seeds (GNS) to the diets of fattening pigs on growth performance, carcass characteristics, and the physicochemical properties of the meat. In the growing and finishing I phases, 32 and 31 pigs, respectively, were used to evaluate five dietary treatments: control diet (T1), control diet + antibiotic (oxytetracycline, T2), control diet + 0.1% GNS (T3), control diet + 0.2% GNS (T4), and control diet + 0.3% GNS (T5). In the finishing II phase, 13 pigs were used to evaluate T1, T2, and T3, and the physicochemical properties of the meat from these pigs were determined. The study used a completely randomised, unbalanced experimental design. In growing pigs, 0.2% GNS reduced feed intake, while the other growth performance variables and carcass characteristics were not affected by the level of GNS inclusion. In the finishing I phase, 0.3% GNS reduced feed intake and the feed-to-gain ratio; however, there were no significant effects on the other measured variables. In finishing II pigs, GNS supplementation did not modify growth performance; however, 0.1% GNS modified the meat pH, L*, and a* values. Ground neem seeds at higher inclusion levels may improve the feed efficiency of pigs, but may also have a negative effect on some meat quality parameters.

Keywords: antibiotic, carcass, feed alternative, meat quality

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Introduction

In pig production, antibiotics have generally been used to maintain the health of animals and increase their productivity. A recent estimate suggests that by 2030, the global use of antibiotics for the promotion of growth in animal production will be 11.5% higher than in 2017 (Tiseo *et al*., 2020). However, the excessive use of antimicrobials represents a risk to global human health as well as the environment because of the collateral effects of this use (Allel *et al*., 2023).

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The swine industry is an important agricultural sector worldwide because of the high human intake of pork; however, it is also a source of concern because of its use of antibiotics. Environmental contamination with antibiotic residues from swine farm wastewater could have long-term effects on the surrounding surface water and groundwater and could contribute to the widespread distribution of various antibiotic resistance genes (Ma *et al*., 2025). These concerns about antibiotic use have led to increased interest in the use of herbal growth promoters as alternatives to antibiotics in animal nutrition, as they have the potential to maintain the efficiency of production while also protecting human health and the environment (Mafouo *et al*., 2019).

Neem (*Azadirachta indica*) is used in ethnoveterinary medicine by rural pig farmers to treat worm infestations (Doley *et al*., 2022), and aqueous neem fruit extracts have acaricidal effects against mange mites and can provide a cheaper, safer, and more eco-friendly alternative for the control of mange in pigs (Pasipanodya *et al*., 2021). Neem also contains phytochemicals with diverse biological and pharmacological activities (Rahmani *et al*., 2018), making it relevant as a possible growth promoter. Previous studies have found that adding neem to pig (Sastry & Agrawal, 1992), chicken (Trigueros *et al*., 2015; Paul *et al*., 2020), and rabbit (Unigwe *et al*., 2016) diets improved their growth performance and carcass characteristics; however, excessive doses reduced these variables because of the bitter flavour of neem and because it also contains toxic substances (Ogbuewu *et al*., 2011). Reports (Sastry & Agrawal, 1992; Annongu *et al*., 2003; Sokunbi *et al*., 2024) also suggest that treated whole neem kernels can be a suitable protein feedstuff for swine at inclusion levels of 10% to 20% of the diet.

We hypothesised that dietary neem supplementation could contribute to the reduction of antibiotic use as growth promoters in the swine industry without affecting the quality of the meat produced, because of the existing evidence that dietary neem shows metabolic activity as a growth promoter. This study thus evaluated the effects of adding ground neem seeds (GNS) to fattening pig diets on the growth performance, carcass characteristics, and physicochemical properties of pig meat to determine whether neem is a viable alternative to antibiotics for promoting growth in pigs.

Materials and methods

The experiment was conducted according to the Rules for the Use and Care of Animals Destined for Research of the Colegio de Postgraduados, as approved by the General Academic Council of the same institution (Regulatory Agreement 04/09/19), following the directives of the Official Rules and Norms of Mexico.

The research was conducted at the Swine Unit of the Experimental Farm of the Colegio de Postgraduados, located in Montecillo, Texcoco, State of Mexico (19° 48' 23" N, 98° 48' 27" W, at 2241 m above sea level). The regional climate is temperate subhumid, with an average temperature of 15.2 °C and an annual rainfall of 644.8 mm. Most rain occurs in summer, with winter rainfall of less than 5% (García, 2004).

Animals, facilities, and treatments

The neem fruits were collected during the spring of 2019; they were soaked in water for 24 hours and the epicarp and mesocarp were then removed by hand. The seeds were washed and dried in the shade for a week, before being dried in a convection oven (Felisa®, model 293A, Mexico) at 100 °C for 24 hours to reduce the moisture content. The dried seeds were then ground in a mill (Thomas, model 4 Wiley®, USA) and passed through a 1 mm screen, producing the GNS used in this study.

Before the start of the study, all the pigs received a prophylactic treatment of 10 mg Ivermectin, 500 000 IU vitamin A, 75 000 IU vitamin D, 50 IU vitamin E, and 1 mL vehicle. The study consisted of three experimental periods: the growing phase, the finishing I phase, and the finishing II phase. In the growing phase, 32 hybrid pigs (10 gilts and 22 barrows; Landrace × Yorkshire × Pietrain) with an average initial body weight of 27 ± 2.89 kg were used to evaluate five treatments: T1 (control diet without antibiotic, n = 5), T2 (T1 + 0.1% oxytetracycline, n = 5), T3 (T1 + 0.1% GNS, n = 5), T4 (T1 + 0.2% GNS, n = 6), and T5 (T1 + 0.3% GNS, n = 6). The growing phase evaluation period lasted five weeks. For the finishing I phase, 31 hybrid pigs with an average initial body weight of 44.33 ± 4.97 kg were used to evaluate the same treatments as in the growing phase (T1: n = 5, T2: n = 5, T3: n = 5, T4: n = 6, and T5: n = 5). This evaluation period also lasted five weeks. Because no clear effects of the dietary treatments were found for the majority of the measured variables in the growing and finishing I phases, it was decided that only the lower GNS inclusion level would be used in the finishing II phase. Thus, in

the finishing II phase, 13 pigs with an average initial body weight of 69.41 ± 5.27 kg were selected to evaluate three treatments: T1 (control diet without antibiotic, n = 4), T2 (T1 + oxytetracycline, n = 4), and T3 (T1 + 0.1% GNS, n = 5). This phase lasted six weeks.

The experimental diets were formulated using the Solver command of Microsoft Office Excel (2016), to meet the National Research Council (2012) requirements for pigs during the three stages of fattening (Table 1).

Table 1 Experimental diets for growing, finishing I, and finishing II pigs

Ingredients (%)	Control diets		
	Growing	Finishing I	Finishing II
Maize grain	77.87		81.24
Sorghum grain		83.87	
Soya bean meal (44% crude protein)	18.66	12.36	16.00
Soya bean oil	0.39	0.84	0.02
L-lysine (54.6%)	0.44	0.65	0.25
DL-methionine (99%)	0.05	0.12	0
L-threonine	0.07	0.10	0.05
L-tryptophan (98%)	0.10	0.07	0.01
Mineral premix[1]	0.15	0.15	0.15
Vitamin premix[2]	0.20	0.20	0.20
Antibiotic[3]	0	0	0
Calcium carbonate	1.49	1.06	0.48
Choline chloride[4]	0.15	0.15	0.15
Sodium chloride	0.30	0.30	0.30
Neem	0	0	0
Protease[5]	0.03	0.03	0.01
Mycotoxin sequestrant[6]	0.10	0.10	0.30
Chemical composition (calculated %, unless otherwise indicated)			
Metabolisable energy (Mcal/kg)	3.30	3.30	3.30
Crude protein	16	14.50	14
Arginine	0.90	0.68	0.73
Phenylalanine	0.70	0.63	0.58
Histidine	0.40	0.30	0.34
Isoleucine	0.58	0.51	0.46
Leucine	1.39	1.33	1.15
Lysine	1.00	0.85	0.73
Methionine + cystine	0.55	0.48	0.43
Methionine	0.29	0.32	0.21
Threonine	0.60	0.52	0.46
Tryptophan	0.17	0.15	0.13
Valine	0.76	0.64	0.51
Calcium	0.66	0.66	0.52
Phosphorus	0.35	0.33	0.24

[1] Rekavit 2000 Fattening Pigs: iron, zinc, manganese, copper, selenium, iodine, and chromium. [2] Rekavit 2000: retinol, cholecalciferol, alpha-tocopherol, menadione, thiamine, riboflavin, pyridoxine, niacin, choline, cyanocobalamin, pantothenic acid, and biotin. [3] Terramycin® (oxytetracycline and thiamine). [4] Kolvit® (60%). [5] Poultrygrow 250™ dehydrated soluble extract of *Streptomyces griseus* fermentation. [6] Adsorsil S (natural zeolite combined with *Yucca schidigera* extract).

The experimental diets were formulated to contain equal amounts of metabolisable energy and protein, and contained maize, sorghum grain, soya bean meal, soya bean oil, crystalline amino acids, vitamin and mineral premix, mycotoxin sequestrant, and protease. The diet was offered in a meal form and the pigs had *ad libitum* access to feed and water throughout the trial.

During the experiment, each pig was allotted to a single metal pen (1.2 × 1.5 m) with a concrete floor partly covered by plastic slats, and equipped with a Hopper-type metal feeder, a drinker, and a 250 W incandescent lamp. The pens were cleaned and the pigs' health inspected daily.

Growth performance and carcass characteristics

The following growth performance variables were measured: average daily feed intake (ADFI, kg/d), average daily gain (ADG, kg/d), feed-to-gain ratio (FGR, kg:kg), fat-free lean gain (FFLG, g/d), and initial and final body weight (BWi and BWf, kg). The following carcass characteristics were measured: initial and final *Longissimus lumborum* muscle area (LMAi and LMAf, cm^2), initial and final backfat thickness (BFTi and BFTf, mm), and initial and final lean meat percentage (LMPi and LMPf, %). These variables were measured at the beginning and end of each experimental period. The LMA and BFT were measured between the first and second lumbar vertebrae on the right side of the pig, using a real-time ultrasound (Medison, SonoVet 600, USA). Using these data, the FFLG, LMPi, and LMPf were estimated using procedure number 5 of Burson & Berg (2001), to estimate the carcass composition of the pigs.

Physicochemical characteristics of the meat

The pigs were slaughtered at the end of the finishing II phase (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, 2014). Meat samples were taken from the *L. lumborum* muscles and immediately stored at 4 °C. At 48 hours post mortem, the colour, pH, and water-holding capacity (WHC) were measured. During these evaluations, the samples were kept on aluminium trays at ambient temperature.

To measure the pH, a portable potentiometer was used (Hanna®, model HI 99163, Spain). The meter was calibrated using standard solutions at pH 4 and 7 (± 0.02 at 20 °C) prior to taking the measurements. The pH was measured three times at three points along the *L. lumborum* samples.

The WHC was determined using the procedure described by Pérez & Ponce (2013). The results were expressed as the volume (in mL) of 0.6 M sodium chloride solution retained by 100 g of meat. The analysis was performed in triplicate.

The colour of the meat was evaluated using a portable colorimeter (Konica Minolta, model CR-410, USA) and was expressed using the L* (brightness), a* (red/green), and b* (yellow/blue; Roberson, 1990) coordinates. The colour was measured in three adjacent positions on the meat sample, so as to obtain a representative measurement of the entire surface of the meat sample.

Statistical analysis

The experimental design used in the three stages of fattening was completely randomised and unbalanced, with each pig in a single pen being considered an experimental unit. The normality and homogeneity of variance of the data were evaluated using the Shapiro-Wilk and Levene's and Bartlett's tests, respectively, with a significance level of $P \leq 0.05$. When the design assumptions were confirmed, the data were analysed using analysis of variance (ANOVA) and analysis of covariance (ANCOVA). In the latter case, the BWi, BFTi, LMAi, and LMPi (which were not response variables) were used as covariates in the statistical analysis. There were no effects of these covariates, so when significant differences were indicated by the ANOVA, the treatment means were compared using the Tukey ($P \leq 0.10$) test. The variables that did not fit with the assumptions of the design were analysed using the Kruskal-Wallis non-parametric test ($P = 0.10$). All procedures were performed using the Statistical Analysis System for Windows (SAS Institute Inc., 2009).

Economic viability

To compare the economic efficiencies (Bellaver *et al.*,1985) of the tested diets, the feeding cost per kilogram of live pig produced was determined using the following equation:

$$Y_i = \frac{Q_i * P_i}{G_i}$$

where:
Y_i = the average cost of feed per kilogram of live weight produced in the treatment,
Q_i = the quantity of feed consumed by the pigs in the treatment group,
P_i = the price of the feed consumed in the treatment group, and
G_i = the weight gain of the pigs in the treatment group, verified for the period.

The economic efficiency index and cost index (Fialho *et al.*, 1992) were then determined using the following formulae:

$$EEI = \frac{MC}{CT_i} \times 100$$

$$CI = \frac{CT_i}{MC} \times 100$$

where:
EEI = the economic efficiency index,
CI = the cost index,
MC = the lowest diet cost/kg of weight gain observed among the treatments, and
CT_i = the cost of the treatment considered.

Results and discussion

The results of the growing stage are presented in Table 2. In pigs fed 0.2% GNS, the ADFI was reduced by 19.19% compared to the pigs fed the control diet ($P \leq 0.10$). However, the other growth performance variables (ADG, FGR, FFLG, and BWf) and carcass characteristics (BFTf, LMAf, and LMPf) were not affected by the level of GNS inclusion or the antibiotic in the diet ($P > 0.10$).

The results for the finishing I stage are presented in Table 3. The addition of GNS or oxytetracycline to the feed did not affect the ADG, FFLG, or BWf of the pigs ($P > 0.10$); however, the ADFI and FGR differed between the treatments ($P \leq 0.10$). The ADFI was lower in the pigs fed the GNS diets and was higher in the pigs fed the control diet: the pigs fed 0.3% GNS consumed 22% less feed than the pigs fed the control diet. Correspondingly, the highest FGR was observed in the pigs fed the control and antibiotic diets, and feeding the pigs 0.3% GNS improved this variable, resulting in FGR values that were 14% and 12% less than those of the control and antibiotic treatment groups, respectively ($P \leq 0.10$). The BFTf, LMAf, and LMPf were not significantly affected by the addition of GNS or antibiotics to the pigs' diets. There were also no trends of change ($P > 0.10$) in these variables in the finishing I pigs in response to GNS or antibiotic supplementation.

In the finishing II pigs (Table 4), the supplementation of GNS or antibiotics in the diet did not modify the ADFI, ADG, FGR, FFLG, or BWf ($P > 0.10$). In addition, the BFTf, LMAf, and LMPf were not affected ($P > 0.10$) by the supplementation of GNS or oxytetracycline.

The results for the physicochemical characteristics of the meat (Table 5) indicated that the meat pH of the pigs fed GNS was lower than that of the pigs fed the diet containing antibiotics ($P \leq 0.10$), and that both of these treatments did not differ from the control diet. However, despite these differences in the pH ($P \leq 0.10$), there were no significant differences between the treatments in the WHC of the meat samples.

The meat of the pigs fed GNS had higher L* values than that of the pigs fed the control and antibiotic-containing diets ($P \leq 0.10$). In addition, GNS supplementation reduced the a* value relative to that of meat from the pigs fed the antibiotic diet ($P \leq 0.10$); however, neither treatments differed from the control diet for this variable ($P > 0.10$). The b* values were similar for all the treatments ($P > 0.10$).

Table 2 The effects of dietary supplementation with ground neem seeds (GNS) or antibiotics on the growth performance and carcass characteristics of growing pigs (mean ± standard deviation)

Treatments	GNS (%)	Antibiotics (%)	Growth performance					Carcass characteristics		
			ADFI (kg/d)	ADG (kg/d)	FGR	FFLG (g/d)	BWf (kg)	BFTf (mm)	LMAf (cm^2)	LMPf (%)
1	0	0	1.72^{a} ± 0.23	0.59 ± 0.06	2.87 ± 0.24	216 ± 37.71	47.47 ± 5.02	10.00 ± 1.07	21.20 ± 3.28	30.27 ± 0.72
2	0	0.1	1.70^{ab} ± 0.16	0.58 ± 0.06	2.90 ± 0.28	225 ± 32.15	45.96 ± 5.30	9.00 ± 1.29	21.55 ± 2.88	30.86 ± 1.15
3	0.1	0	1.55^{ab} ± 0.12	0.54 ± 0.03	2.92 ± 0.14	201 ± 35.49	44.75 ± 4.09	9.00 ± 1.26	19.44 ± 4.49	29.65 ± 1.69
4	0.2	0	1.39^{b} ± 0.23	0.53 ± 0.06	2.64 ± 0.14	212 ± 16.19	44.25 ± 4.28	9. 17 ± 0.75	21.20 ± 1.12	31.06 ± 1.42
5	0.3	0	1.50^{ab} ± 0.19	0.54 ± 0.05	2.61 ± 0.28	219 ± 21.21	44.55 ± 4.80	8.40 ± 1.14	20.71 ± 3.04	31.01 ± 0.67
***P*-value**			0.06	0.14	0.14	0.71	0.70	0.16	0.78	0.22
CV			12.57	9.82	8.03	14.30	10.45	12.22	15.14	3.91
MSD			0.33	0.08	0.39	45.17	6.99	1.64	4.63	1.77
SEM			0.19	0.05	0.22	30.77	4.76	1.12	3.15	1.19
R^2			0.34	0.21	0.30	0.07	0.07	0.20	0.05	0.19

[a,b] Treatment means with different superscripts in each column indicate significant differences ($P \leq 0.10$). T1: control diet, T2: control diet + 0.1% oxytetracycline, T3: control diet + 0.1% GNS, T4: control diet + 0.2% GNS, T5: control diet + 0.3% GNS. ADFI: average daily feed intake, ADG: average daily gain, FGR: feed:gain ratio, FFLG: fat-free lean gain, BWf: final body weight, BFTf: final backfat thickness, LMAf: final *Longissimus lumborum* muscle area, LMPf: final lean meat percentage, CV: coefficient of variation, MSD: minimum significant difference, SEM: standard error of the mean, R^2: coefficient of determination.

Table 3 The effects of dietary supplementation with ground neem seeds (GNS) or antibiotics on the growth performance and carcass characteristics of finishing I pigs (mean ± standard deviation)

Treatments	GNS (%)	Antibiotics (%)	Growth performance					Carcass characteristics		
			ADFI (kg/d)	ADG (kg/d)	FGR	FFLG (g/d)	BWf (kg)	BFTf (mm)	LMAf (cm^2)	LMPf (%)
1	**0**	**0**	2.34^{a} ± 0.19	0.69 ± 0.04	3.39^{a} ± 0.14	284 ± 36.21	71.25 ± 5.51	11.00 ± 1.22	28.84 ± 1.87	30.19 ± 0.78
2	**0**	**0.1**	2.07^{ab} ± 0.22	0.60 ± 0.08	3.30^{a} ± 0.25	253 ± 36.13	65.38 ± 9.95	10.50 ± 1.05	28.67 ± 3.99	30.79 ± 0.42
3	**0.1**	**0**	2.03^{ab} ± 0.25	0.65 ± 0.06	3.07^{ab} ± 0.16	260 ± 39.38	65.94 ± 5.99	10.13 ± 0.99	26.97 ± 2.97	30.04 ± 0.66
4	**0.2**	**0**	2.08^{ab} ± 0.42	0.64 ± 0.10	3.15^{ab} ± 0.15	255 ± 47.40	66 ± 6.79	9.83 ± 0.98	27.46 ± 1.83	30.45 ± 0.67
5	**0.3**	**0**	1.82^{b} ± 0.24	0.61 ± 0.08	2.89^{b} ± 0.46	248 ± 28.77	65.79 ± 4.58	10.5 ± 1.22	28.86 ± 2.34	30.81 ± 0.53
***P*-value**			0.09	0.36	0.04	0.58	0.60	0.46	0.61	0.12
CV			13.52	11.74	7.59	14.73	10.20	10.49	9.86	2.01
MSD			0.43	0.11	0.40	57.06	10.15	1.62	4.13	0.94
SEM			0.27	0.07	0.24	38.23	6.80	1.08	2.76	0.61
R^2			0.27	0.14	0.37	0.10	0.09	0.12	0.09	0.24

[a,b] Treatment means with different superscripts in each column indicate significant differences ($P \leq 0.10$). T1: control diet, T2: control diet + 0.1% oxytetracycline, T3: control diet + 0.1% GNS, T4: control diet + 0.2% GNS, T5: control diet + 0.3% GNS. ADFI: average daily feed intake, ADG: average daily gain, FGR: feed:gain ratio, FFLG: fat-free lean gain, BWf: final body weight, BFTf: final backfat thickness, LMAf: final *Longissimus lumborum* muscle area, LMPf: final lean meat percentage, CV: coefficient of variation, MSD: minimum significant difference, SEM: standard error of the mean, R^2: coefficient of determination.

Table 4 The effects of dietary supplementation with ground neem seeds (GNS) or antibiotics on the growth performance and carcass characteristics of finishing II pigs (mean ± standard deviation)

Treatments	GNS (%)	Antibiotics (%)	Growth performance					Carcass characteristics		
			ADFI (kg/d)	ADG (kg/d)	FGR	FFLG (g/d)	BWf (kg)	BFTf (mm)	LMAf (cm^2)	LMPf (%)
1	0	0	3.53 ± 0.41	0.91 ± 0.11	3.92 ± 0.72	317 ± 51.40	107.50 ± 9.35	13.25 ± 2.06	36.23 ± 6.23	28.63 ± 0.78
2	0	0.1	3.44 ± 0.43	0.93 ± 0.11	3.72 ± 0.56	316 ± 48.42	107.30 ± 7.54	14.40 ± 1.52	35.91 ± 5.18	28.28 ± 0.89
3	0.1	0	3.34 ± 0.24	0.94 ± 0.07	3.57 ± 0.37	334 ± 21.71	110.88 ± 6.28	14.50 ± 1.29	38.26 ± 3.09	28.60 ± 0.89
***P*-value**			0.77	0.93	0.69	0.79	0.76	0.50	0.76	0.78
CV			10.98	10.86	15.13	13.44	7.18	11.66	13.68	2.99
MSD			0.59	0.15	0.89	68.34	12.31	2.59	7.94	1.34
SEM			0.37	0.10	0.56	43.26	7.79	1.64	5.02	0.85
R^2			0.04	0.01	0.07	0.04	0.05	0.12	0.05	0.04

[a,b] Treatment means with different superscripts in each column indicate significant differences ($P \leq 0.10$). T1: control diet, T2: control diet + 0.1% oxytetracycline, T3: control diet + 0.1% GNS, T4: control diet + 0.2% GNS, T5: control diet + 0.3% GNS. ADFI: average daily feed intake, ADG: average daily gain, FGR: feed:gain ratio, FFLG: fat-free lean gain, BWf: final body weight, BFTf: final backfat thickness, LMAf: final *Longissimus lumborum* muscle area, LMPf: final lean meat percentage, CV: coefficient of variation, MSD: minimum significant difference, SEM: standard error of the mean, R^2: coefficient of determination.

Table 5 The effects of dietary supplementation with ground neem seeds (GNS) or antibiotics on the physicochemical properties of the *Longissimus lumborum* meat of pigs (mean ± standard deviation)

Treatments	GNS (%)	Antibiotics (%)	Colour			Water-holding capacity (%)	pH
			L*	a*	b*		
1	0	0	$54.69^{b} \pm 3.17$	$16.97^{ab} \pm 1.07$	5.32 ± 0.98	24.67 ± 4.46	$5.57^{ab} \pm 0.15$
2	0	0.1	$54.48^{b} \pm 2.26$	$17.71^{a} \pm 1.15$	5.15 ± 0.42	23.17 ± 3.66	$5.64^{a} \pm 0.12$
3	0.1	0	$57.65^{a} \pm 1.93$	$16.61^{b} \pm 1.12$	5.46 ± 0.53	23.00 ± 3.77	$5.47^{b} \pm 0.04$
***P*-value[1]**			0.01	0.06		0.53	0.003
***P*-value[2]**					0.37		
CV			4.50	6.49		16.85	2.01
MSD			2.51	0.96		3.98	0.11
SEM			2.50	1.11		3.97	0.11
R^2			0.26	0.15		0.03	0.29

[a,b] Treatment means with different superscripts in each column indicate significant differences ($P \leq 0.10$). T1: control diet, T2: control diet + 0.1% oxytetracycline, T3: control diet + 0.1% GNS. [1] Pr > F of analysis of variance, [2] Pr > Chi-squared of Kruskal-Wallis test. L*: luminosity, a*: red index, b*: yellow index, CV: coefficient of variation, MSD: minimum significant difference, SEM: standard error of the mean, R^2: coefficient of determination.

Neem is considered one of the most promising plant species of the 21st century (Ogbuewu *et al*., 2011) because of the many beneficial properties its products have been found to have. Neem products are active against both Gram-positive and Gram-negative bacteria (Sharma & Srivastva, 2014); inhibit the growth of *Trichophyton*, *Microsporum*, and *Epidermophyton* dermatophytes (Ospina *et al*., 2015); have anthelmintic activity against *Pheretima posthuma*, *Ascaridia galli*, *Raillietina spiralis* (Rabiu & Subhasish, 2011), *Paramphistomum cervi*, and *Fasciola hepatica* (Ibekwe, 2019); reduce blood glucose levels in a short period of time (Nagashayana *et al*., 2014); eliminate free radicals (Sithisarn *et al*., 2005); and have the potential to prevent and treat a great number of human cancers (Patel *et al*., 2016).

In animal nutrition, broilers fed ground neem leaves had higher final weights and feed efficiencies than broilers fed control and Terramycin-containing diets (Ansari *et al*., 2012). The addition of ground neem leaves to broiler and rabbit feeds has thus been recommended because it is not harmful and helps improve animal growth performance (Unigwe *et al*., 2016; Deka *et al*., 2019). Similarly, GNS included in a broiler diet was found to optimise the ADG, ADFI, and FGR (Trigueros *et al*., 2015). These previous findings for broilers and the digestive and nutrient absorptive similarities between pigs and chickens led to the hypothesis at the beginning of this study that at least one of the levels of GNS inclusion would improve the growth performance of the pigs. However, this was not the case, with only the ADFI and FGR differing between the treatments. These conflicting results may be because of the different domestic animals and parts of the tree (leaves versus seeds) used in this study compared to the previous studies. In addition, the effect of neem depends on the level at which it is used and the dose-response relationship, and the different results found may thus have been due to the different levels of supplementation used.

Higher levels of GNS in the diet (0.2% and 0.3% in the growing and finishing I stages, respectively) interfered with the pigs' acceptance of the feed. These results agree with those of Trigueros *et al*. (2015), who indicated that higher levels of GNS inclusion in broiler diets (3% and 5%) reduced feed intake. Ubua *et al*. (2019) similarly reported lower ADFI values for a diet containing 7.5% ground neem leaves during the entire broiler fattening period. In contrast, the supplementation of lower doses of ground neem leaves (0.2% and 0.3%) to broilers improved their feed intake (Deka *et al*., 2019). In relation to these results, neem products are known to contain two main types of phytochemicals: isoprenoids and non-isoprenoids (Gupta *et al*., 2017). Some of these compounds, such as azadirachtin, meliacin, gedunin, salanin, nimbin, and valassin, are responsible for the bitter flavour and strong aroma of neem, which may interfere with animals' acceptance of feeds containing neem (Aruwayo *et al*., 2011).

In the finishing I phase (but not the other two phases), the FGR was lower in the pigs fed the diet containing the highest concentration of GNS (0.3%). This concurs with the reports of Sastry & Agrawal (1992), who found that the efficiency of dry matter utilisation improved when 10% neem seed meal washed with water was supplemented in a pig diet. Likewise, the FGR of broilers or rabbits improved with the inclusion of ground neem leaves, GNS, or an aqueous extract of neem leaves in the diet (Trigueros *et al*., 2015; Unigwe *et al*., 2016; Deka *et al*., 2019; Paul *et al*., 2020).

The improvement in FGR observed can be attributed to the antibacterial and anthelmintic potential of neem seeds. The main mode of action of herbal additives is modulating the gastrointestinal microbiome, which competes with the animal and can cause inflammation in the intestinal epithelium by producing toxins (Liu *et al*., 2011). Consequently, modulating the microbiome can decrease intestinal inflammation and enhance feed digestion and absorption, resulting in improvements in the feed conversion efficiency and weight gain (Sarker *et al*., 2014). In addition, pigs can adapt to feedstuffs with poor palatability, up to a point, and feed additives such as GNS can thus be used to control feed intake and, in consequence, improve digestion and the FGR.

However, in this study there were no effects of GNS on the ADG, FFLG, or BWf across the entire fattening period. The results for the BWf are consistent with the findings of Egbeyale *et al*. (2020), who reported that the supplementation of ground neem leaves to broilers at 0.5%, 1.0%, or 1.5% had no effect on the birds' body weights. In contrast, Sastry & Agrawal (1992) improved the ADG of pigs by adding washed neem seed meal to the diet. Other authors have similarly reported improvements in body weight in response to supplementing 2.5 g of ground neem leaves per kilogram of feed or 1% aqueous extract of neem leaves to broilers' diets (Ansari *et al*., 2012; Paul *et al*., 2020). The contrasting results in the literature and in this study may have been caused by the varied concentrations of neem used, as well as the varied treatments applied to the seeds. These factors can determine the concentrations of phytochemicals, macrominerals, and microminerals in the feed additive (Boeke *et al*., 2004). It may

therefore be expected that unprocessed neem products, such as leaves and seeds included at low levels, may have a less acute effect or may require more time to affect an animal response, relative to when extracts or meals are used (Sarker *et al*., 2014).

The GNS included in the diets had neither positive nor negative effects on the pigs' carcass characteristics, which may be attributed to the effective use of the feed by the pigs. In contrast, Singh *et al*. (2014) included ground neem leaves in guinea hens' feed, and observed that it reduced the serum profile of low-density lipoproteins, triglycerides, and abdominal fat, and increased the serum profile of high-density lipoproteins. This may be associated with the hypocholesterolaemic effect of neem, as Duangjai *et al*. (2019) observed that an extract of neem flowers inhibited the activity of the pancreatic cholesterol esterase enzyme, decreased the uptake of cholesterol by Caco-2 intestinal cells, and inhibited the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, key factors in cholesterol biosynthesis. This resulted in a decreased accumulation of cholesterol in the muscles.

As was the case for the growth performance variables, the doses of GNS used in this study may not have been sufficient to modify the carcass characteristics or reduce backfat thickness. Similarly, feeding broilers a diet including ground neem leaves at 0.5%, 1%, 1.5%, 2.5%, 5%, or 7.5% generally had no effect on carcass variables (Ubua *et al*., 2019), apart from on the weight of the back portion of carcass, which was highest in the 1% ground neem leaves treatment group (Egbeyale *et al*., 2020). Mafouo *et al*. (2019) did not find significant differences between the carcass characteristics of broilers fed a diet supplemented with neem seed oil (15, 20, or 25 g/kg), and those of broilers fed the control diet or a doxycycline-containing diet. In contrast, Ansari *et al*. (2012) and Paul *et al*. (2020) reported better carcass characteristics when broilers were fed diets supplemented with ground neem leaves or an aqueous extract of neem leaves than when they were fed a control diet, possibly reflecting an improvement in the growth performance of the birds.

Both antibiotics and neem products can control the growth and colonisation of several diverse pathogenic and non-pathogenic microorganisms in the animal intestine. A more balanced microbiome in the gastrointestinal tract means higher digestive efficiency and feed utilisation, improving growth rate and feed efficiency (Costa *et al*., 2011; Sarker *et al*., 2014). However, in this experiment, the growth performance and carcass characteristics did not benefit from the dietary antibiotic, because the results for this treatment group did not differ from those of the control group. These results are consistent with those of Landy *et al*. (2011), who reported that dietary flavophospholipol had no positive effects on the yield and carcass cuts of broilers, compared to the control diet. Lowell *et al*. (2018) similarly found no differences between a control diet and diets supplemented with tylosin or oregano oil in a study examining the growth performance and carcass characteristics of pigs.

The results for the pigs fed any level of GNS (0.1%, 0.2%, or 0.3%) in this study did not significantly differ from those of the pigs fed the antibiotic-containing diet (T2). Similarly, there were no significant differences in body weight and feed efficiency in chickens when they consumed diets containing Terramycin or 1.5 and 5 g ground neem leaves per kilogram of feed (Ansari *et al*., 2012). Similar results were reported for chickens fed a diet containing an aqueous extract of neem leaves (Paul *et al*., 2020). In contrast, Mafouo *et al*. (2019) reported that feeding broilers diets supplemented with neem seed oil reduced their body weight and weight gain, and increased their feed conversion ratio, compared to broilers fed a doxycycline-containing diet. In addition, Landy *et al*. (2011) reported an improvement in the body weights of broilers fed a flavophospholipol diet, compared to broilers fed 7 g or 12 g of neem flower meal per kilogram of feed, without differences in daily feed intake between the treatments. The lack of a growth promotor effect found in this study may be related to the diet digestibility or the environmental conditions. Generally, growth promoters have a greater effect when the diet is less digestible, and healthy and well-fed animals do not respond to such feed additives when they are housed under good sanitary conditions at a moderate population density (Lowell *et al*., 2018).

It has been previously observed that the addition of antibiotics to pig diets does not affect the physicochemical properties of the meat (Lowell *et al*., 2018), which concurs with the findings of this study. Likewise, ground neem leaves did not modify the pH of broiler meat (Egbeyale *et al*., 2020). In contrast, Killi *et al*. (2015) found that including ground neem leaves at 0.2% in a broiler diet significantly increased the pH of both the fresh meat and the meat after storage for a week compared to the meat of chickens fed an oxytetracycline-containing diet, but that this parameter was lower than in the meat of broilers fed the control diet. In the current study, the meat of the pigs fed the GNS-supplemented diets had lower pH values than that of the pigs fed the antibiotic-containing diet.

Usually, the pH of muscle decreases from 7.0–7.2 (physiological pH) to 5.5–5.8 during the first

24 to 48 hours after slaughter (Brewer *et al*., 2001). Chmiel *et al*. (2016) notes that the ideal pH for meat is higher than 5.5, thus, in this experiment, the meat from the pigs fed the GNS-supplemented diets was slightly below the pH range for normal meat, indicating better glycogen reserves in the muscles of these pigs. However, as a general rule, a low pH has a harmful effect on the quality characteristics of pig meat, because the combination of a high temperature and a low pH (close to the isoelectric point for muscle proteins) favours the denaturation of proteins and the damage of cell membranes. In consequence, the WHC of the meat deteriorates (Chmiel *et al*., 2016). Nonetheless, the WHC did not change in response to dietary GNS supplementation in this study; however, the lack of subsequent measurements could have prevented our observation of the effects of the low pH on the WHC.

Meat colour is probably the main factor that determines the consumer response to meat and their decision whether to purchase meat. This variable is associated with both the pH and the meat maturation time. An attractive meat colour is one that has low brightness, high red intensity, and low yellow intensity. The addition of GNS to the diet modified the meat colour values, significantly increasing the L* value and decreasing the a* value, resulting in meat with a pale appearance.

In the calculation of the EEI and CI, the lowest feed cost per kilogram of weight gained was considered (Table 6). The control and antibiotic-containing diets exhibited the worst EEI and CI in all three production phases. For the growing and finishing I phases the diets containing 0.2% and 0.3% GNS resulted in better EEI and CI, respectively, whereas in the finishing II phase the diet containing 0.1% GNS achieved the better EEI and CI values. The observed results show that the dietary addition of GNS at 0.1%, 0.2%, and 0.3% enabled the production of pigs at a lower cost, resulting in greater economic viability.

Table 6 Bioeconomic parameters of diets supplemented with ground neem seeds (GNS) or antibiotics

Parameter	Growing stage				
	T1	**T2**	**T3**	**T4**	**T5**
Cost per kg of feed ($)	0.535	0.557	0.538	0.541	0.544
Feed cost/kg of weight gained ($)	1.560	1.633	1.544	1.419	1.511
Economic efficiency index	90.971	86.908	91.878	100.00	93.894
Cost index	109.924	115.064	108.838	100.00	106.502
	Finishing I stage				
	T1	**T2**	**T3**	**T4**	**T5**
Cost per kg of feed ($)	0.509	0.531	0.512	0.514	0.517
Feed cost/kg of weight gained ($)	1.726	1.832	1.599	1.671	1.543
Economic efficiency index	89.360	84.201	96.467	92.339	100.000
Cost index	111.906	118.763	103.662	108.296	100.00
	Finishing II stage				
	T1	**T2**	**T3**		
Cost per kg of feed ($)	0.482	0.504	0.484		
Feed cost/kg of weight gained ($)	1.870	1.864	2.984		
Economic efficiency index	91.978	92.248	100.000		
Cost index	108.721	108.403	100.000		

All amounts are expressed in US dollars ($), and are as of May 2025. T1: control diet, T2: control diet + 0.1% oxytetracycline, T3: control diet + 0.1% GNS, T4: control diet + 0.2% GNS, T5: control diet + 0.3% GNS.

Conclusions

Based on the results of this study, we conclude that GNS included in the diet at high levels (0.2% and 0.3% in the growing and finishing I stages, respectively) improves the feed utilisation of pigs, because feed intake was reduced without affecting the other growth performance variables. However,

dietary GNS may have negative effects on meat quality properties. Additionally, it was observed that neither the antibiotics nor the GNS promoted the growth performance of the pigs, which were reared under good sanitary conditions at a moderate population density. Considering that dietary GNS reduced feed intake without affecting other productive variables, it may be a suitable alternative for antibiotics, both to reduce feed cost and avoid environmental or public health implications.

Authors' contributions

J.L.F.-V., M.Y.V.-P., M.T.S.-T., J.A.M.-A., J.L.G.-C., J.L.C.-M., A.L.-T., and M.M.C.-G. were responsible for the design and execution of the research. All co-authors participated in the management and discussion of the results and statistical analyses, and the writing of the manuscript.

Conflict of interest declaration

The authors declare that there is no conflict of interest regarding the publication of this article.

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