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RESEARCH

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Antibacterial effects of nano-emulsified cumin oil on performance and carcass characteristics in weaning rabbits infected by *Clostridium perfringens* type A

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Abstract

Background This study aimed to evaluate the antibacterial effects and growth-promoting potential of nano-emulsified cumin oil (NECO) in vitro and in vivo trials using newly weaned Gabaly rabbits. NECO was tested as both a preventive and curative agent against *Clostridium perfringens* type A infection through dietary supplementation.

Methods The study included two experimental phases: (1) an in vitro trial to determine the bacterial inhibition potential of different NECO concentrations (0.1, 0.2, 0.5, and 1 mL) and (2) an in vivo trial using 120 four-week-old newly weaned male Gabaly rabbits (0.544 kg average body weight) allocated into five groups ($n = 24$/group were divided into 3 replicates, 8 animals each). The groups were: G1 (negative control, no infection or supplement), G2 (NECO control, supplemented with 10 mL/kg diet), G3 (positive control, infected with *C. perfringens* type A, no supplement), G4 (preventive NECO, supplemented with 10 mL/kg diet before infection), and G5 (curative NECO, supplemented with 10 mL/kg diet after infection). NECO was formulated as a stable nano-emulsion using a high-shear homogenizer.

Results The in vitro study demonstrated that NECO inhibited *C. perfringens* growth at all tested concentrations, with complete inhibition observed at 1 mL. Based on this, the 1 mL concentration was selected for in vivo trials. Infected rabbits (G3) exhibited clinical signs including anorexia, depression, severe diarrhea, and bloat, with a morbidity rate of 90% and a 35% mortality rate by the 5th day post-challenge. NECO supplementation significantly improved ($p < 0.05$) final body weight (FBW), total weight gain, and average daily gain (ADG) in G2 compared to G3. While G4 and G5 did not significantly differ in FBW or ADG from the control (G1), all NECO-supplemented groups had improved feed conversion ratio (FCR) compared to G3 ($p < 0.05$). The best FCR was observed in G2 (3.80), followed by G4 (4.51) and G5 (4.77), while the worst was in G3 (5.31). Histopathological and postmortem findings confirmed reduced disease severity with NECO supplementation.

Conclusion These results suggest that NECO serves as a natural antibacterial alternative, effectively mitigating *C. perfringens* infection while enhancing growth performance.

Keywords Cumin, Essential oil, Nano-emulsion, Performance, Rabbit, *Clostridium*

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1 Background

Rabbits play a crucial role in addressing protein scarcity worldwide due to their high fertility and rapid growth [1]. Rabbit meat is distinct from other types of meat as it contains no uric acid and has low cholesterol levels. Additionally, it is rich in polyunsaturated fatty acids (PUFAs), essential amino acids, vitamins, and minerals, making it a highly nutritious lean protein source [2]. However, high mortality rates during both pre-weaning and post-weaning periods pose a significant challenge to the rabbit industry, particularly impacting low-income populations [3].

One of the major challenges in commercial rabbit breeding is the high incidence of intestinal diseases, which can cause significant mortality rates ranging from 27 to 50% within five to seven weeks after birth [4, 5]. Poultry farms are a major source of *Clostridium perfringens* infections, particularly in broiler chickens, where the bacterium causes necrotic enteritis (NE), leading to significant economic losses. *C. perfringens* type A was prevalent in both healthy and unhealthy broilers, with a higher presence of the Net B toxin gene linked to NE in diseased birds [6]. *Clostridium* species pose a serious veterinary threat to rabbit farming, with *C. perfringens*, *C. difficile*, and *C. butyricum* being particularly problematic [7]. These toxigenic bacteria are responsible for enterotoxemia, a severe condition characterized by diarrhea and sudden death, and have been identified in weaned rabbit farms across various Egyptian governorates [8].

Several investigations have established the occurrence of *C. perfringens* type A, isolated from the livers and caeca of infected rabbits [9]. *C. perfringens* type A is predominantly related with epidemics of mucoid enteropathy in several Egyptian rabbit farms. It was isolated from the caeca of rabbits that died unexpectedly following a short period of illness displaying severe diarrhea [10].

In both humans and animals, *C. perfringens* has been consistently associated with various systemic and intestinal diseases, including diarrhea, enterocolitis, and food poisoning [11]. Notably, different strains of *C. perfringens* can produce over 20 distinct toxins and enzymes, which serve as key virulence factors in the pathogenesis of infections [12]. *C. perfringens* types A, F, and C are commonly associated with human infections, while types B, E, D, and G are primarily significant in veterinary medicine [13].

Antibiotics are widely used to treat bacterial infections; however, the rise of antimicrobial resistance has diminished their effectiveness. The emergence of antibiotic-resistant bacteria poses a serious threat to treatment efficacy, limiting available therapeutic options even for common infections [14].

Concerns have been expressed about the possibility of generating antibiotic resistance through the use of IFA, which could represent a concern to human health [15]. Studies performed into the transmission of antibiotic resistance genes from animals to humans [16]. As a result, major emphasis should be directed to natural substances that display high efficiency against illnesses while generating minimum adverse effects [17]. Given the increasing concern over antibiotic resistance, integrating probiotics into animal diets offers a sustainable strategy to control bacterial infections, including *C. perfringens*, while promoting overall health and productivity [18]. There is an urgent need to identify natural alternatives that are safe for addressing bacterial and fungal health issues in humans and animals. Numerous spices, such as cumin, clove, thyme, oregano, and cinnamon, have substantial antibacterial and antifungal activities [19]. Recent developments in nanotechnology have improved the antibacterial activities of various biologically active materials [20]. Furthermore, nanobiotechnology has become prominent in recent years, especially with bioactive biological materials, such as plant extracts and essential oils [21]. Nano-emulsions (NEs) are oil-in-water emulsions in which the particle sizes ranging from 20 to 200 nm [22]. Nano-emulsion formation using nanotechnology enhances the stability, solubility and bioavailability of the essential oils in water [23]. Nano-emulsion enhances the antibacterial properties of oils and increases the shelf lives of oils, especially in terms of their physical properties. This occurs as a result of increase the surface area due to smaller droplet size of NEs oils when compared to pure oils, thus making them more effective feed additives than antibiotics [24]. Nano-emulsified essential oils have more bioavailable bioactive compounds than pure essential oils and are more effective in rabbit feed which improve the antioxidant and antibacterial properties of the essential oils [25]**.** *Cuminum cyminum* L. is a plant belonging to the family Apiaceae and originating from Southwestern Asia and Eastern Mediterranean. Cumin is a herb as well as spice with an exotic flavor that is used in foods in various parts of the world; studies have shown that *Cuminum cyminum* L. and its essential oil contain antioxidants, antibacterial, antifungal, and medicinal qualities and thus used as a natural food preservative [26]. Some of the main chemical compositions of Cuminum cyminum L. essential oil and nano-emulsion include Cuminaldehyde (27. 99%), o-Cymene, γ-Terpinen (16. 67%), and β-Pinene (9. 35%) [27]. Cumin essential oil nano-emulsification shows antioxidant and antibacterial effects, suggesting its application in the synthesis of new natural antioxidants and antimicrobial agents for various uses [27]. The cumin nano-emulsion contained eleven major constituents, with oleyl oleate as the most abundant at 33.19% [28].

The current study aimed to assess the efficacy of nano-emulsion of cumin oil as antibiotic alternative and growth promoter. The assessment was performed using in vitro and in vivo studies in recently weaned Gabaly rabbits. The oil nano-emulsion used in the study was applied topically by coating the pelleted diets as a preventive and treatment approach against *C. perfringens* type A infection.

2 Materials and methods

2.1 Preparation of NECO

NECO preparation was done by following previously published methods with modifications from [25, 29, 30]. A litter of NECO was prepared by combining 100 ml of cumin essential oil and 100 ml of Tween 80 as a surfactant with 1:1 proportion. It took 20–30 min to homogenize the mixture with a high shear homogenizer of 1500 watts and then slowly added 80% distilled water. This led to the formation of nano-emulsion of 1 ml of raw cumin oil with the emulsifier and water to make 10 ml nano-emulsion cumin oil.

2.2 Characterization of nano-emulsion

2.2.1 Zeta potential

The surface electrical charge of the nano-emulsion was measured using laser Doppler electrophoresis, which made certain that all the emulsion particles had the same charge and to minimize oil particle re-aggregation as much as possible (Santa Barbara, Calif., USA). The components were diluted with deionized water and injected into a capillary cell at 25 °C to determine charge. The zeta potential values were represented in millivolts.

2.2.2 Transmission electron microscopy (TEM)

The structure and shape of the nano-emulsified cumin essential oil were investigated with a transmission electron microscope (FEI-TECNAI G2-20 TWIN, Netherlands). The nano-emulsions were further diluted with deionized water (tenfold and 100-fold) before being applied to 300 mesh copper grids coated with carbon film. After three hours of vacuum drying, the grids were investigated with TEM at 80 kV, which provided a positive picture of the nanoscale size.

2.2.3 Cumin oil nano-emulsion stability index test

To assess the physical stability of the emulsions, gravitational phase separation was investigated, according to [31]. Freshly made NECO (20 ml) was properly sealed and placed in an incubator set to 25 °C. Cylindrical glass tubes (internal diameter 10 mm, height 50 mm) were used to assess the emulsions' physical stability. Over the course of ninety days, the height of the translucent layer that formed at the bottom of the emulsions (HS) was measured once a week to confirm that gravitational phase separation did not occur. The monitoring tests were carried out in duplicate, and the data analysis in Table 1 was based on the average of three different trials. The emulsion stability index (ESI) was calculated using the equation below: ESI% = (HE−HS)/HE * 100.

2.2.4 Bacterial strain

A locally isolated vaccinal *C. perfringens* type A strain was kindly provided by the Anaerobic Vaccines Research Department, VSVRI. The bacteria were cultivated in cooked meat media for 24 h, yielding an infective C. perfringens concentration of 1 mL, which is equivalent to 1×10^9 cfu/mL. This bacterial culture was used for both in vitro and in vivo experiments.

2.2.5 In-vitro study

The *C. perfringens* culture was prepared to undergo experimental infection. The lyophilized C. perfringens strain was cultivated on cooked meat broth, incubated in an anaerobic jar at 37 °C for 24 h, and tested on blood agar plates. Serial double dilutions of the culture were performed in sterile PBS, and viability cell concentration was assessed by colony count on blood agar. The infectious dose was set to 1×10^9 cfu/mL.

The effect of nano-emulsified cumin oil on bacterial growth was investigated using various concentrations (0.1, 0.2, 0.5, and 1 mL) in broth and cooked meat media. C. perfringens was cultivated on cooked meat broth and then incubated at 37 °C in an anaerobic jar for 24 h before being studied on blood agar plates. Nano-emulsified cumin oil was added to the cooked meat broth, which

Table 1 Stability of Nano-emulsified Cumin Oil

Date (Week)	Temperature (°C)	HS (ml)	HE (ml)	ESI %
1	25	0	20	100
2	25	0	20	100
3	25	0	20	100
4	25	0	20	100
5	25	0	20	100
6	25	0	20	100
7	25	0.5	20	97.50
8	25	1	20	95

This table presents the emulsion stability index (ESI) of nano-emulsified cumin oil (NECO) stored at 25 °C over an eight-week period. The ESI values are given as percentages, indicating the stability of the emulsion over time. A slight decrease in stability is observed from the seventh week onwards. HE: initial height of the emulsion layer when freshly prepared; HS: the height of the translucent layer formed at the bottom due to gravitational phase separation was then re-incubated at 37 °C for 24 h before being analyzed on blood agar plates.

2.2.6 *In vivo study*

The feeding trial was conducted at the Center of Animal Research. All procedures used in this study were approved by the relevant ethics committee and complied with institutional guidelines for the care and use of animals in research. The study was conducted under ethical approval number BUFVTM 08-03-23.

A one-week orientation phase preceded the feeding trial to allow for the transition from milk to dry feeding and to prevent post-weaning shock in young rabbits fed barley and dry experimental feed. The growth or feeding trial started in March 2023 and lasted 12 weeks. The feed requirement of the experimental rabbits was adjusted in accordance with the NRC (1977). The experimental animals' weights were assessed every two weeks, and their food requirements were adjusted correspondingly. Fresh water was available to the animals all day.

Table 2 shows how a basal ration was developed to act as a control. In the NECO-supplemented groups, the feed was sprayed directly onto the pelleted rabbit feed, then dried and stored in a clean, and well-ventilated area until used in the experiment. One hundred and twenty newly male weaned Gabaly rabbits (local breed) at one month old, with an average initial weight of 0.544 kg, allocated into five groups (n = 24/group were divided into 3 replicates, 8 animals each), and rabbits were obtained from rabbit farm. The experimental groups were:

> *Group 1 (Negative Control)* had a standard diet without infection or supplements.
> *Group 2 (NECO Control)* received a standard diet supplemented with 10 mL of NECO per kilogram of diet.
> *Group 3 (Positive Control)* was infected with *C. perfringens* and given a standard diet without supplements.
> *Group 4 (Preventive NECO)* received a standard diet supplemented with 10 mL of NECO per kg of diet prior to being infected with *C. perfringens* type A.
> *Group 5 (Curative NECO)* was infected with *C. perfringens* type A and after that fed a standard diet supplemented with 10 mL of NECO per kg of diet.

The animals in group G3, G4, and G5 were orally administered with 1 mL of *C. perfringens* type A suspension (10^9 CFU/mL) for three consecutive days, while the preventive group (G4) received NECO 10 ml/kg diet before infection for 7 days.

All rabbit groups had their clinical sign of infection checked daily with regard to their clinical indication. On the 5th day after infection, three rabbits from each group were euthanized for clinical and pathological examination. Liver and intestinal tissue samples were collected for histopathological assessment of pathological changes as already explained in [32].

The growth performance of the rabbits was determined through daily morning weights of the rabbits before offering them feed or water. The average body weight gain BWG was calculated as the difference between the average final live body weight LBW and the average beginning LBW for a specified period. The daily residual feed was recorded in detail to arrive at the average feed intake of the animals. FCR was then determined as the total feed intake divided to the weight gain, which shows how effectively feed was used for weight gain.

Table 2 Composition and chemical analysis of basal diet for rabbits

Ingredients	(%)
Yellow corn	18
Soybean meal (44%CP)	25
Wheat bran	11
Clover hay	30
Barley	12
Limestone	1.50
NaCl	0.50
Di- Calcium phosphate	2
Total	100
Calculated chemical analyses	
Digestible energy(kcal/kg)	1465.44
CP%	20.42
EE %	1.73
CF%	14.05
Ca%	1.38
Ph%	0.46
Lysine%	1.16

This table shows the ingredients and their percentages in the basal ration fed to the rabbits. It also shows the calculated chemical analysis of the ration, including digestible energy (MJ/kg), crude protein (%), EE (%), crude fiber (%), calcium (%), phosphorus (%), and lysine (%)

2.3 Histopathological examination

Samples of liver and intestinal tissues were collected and were processed through 10% neutral buffered formalin and paraffin embedding. Slides with 5-µm-thick sections were stained with Harris's hematoxylin and eosin. In the liver, histopathological changes were assessed according to congestion, hemorrhage, cytoplasmic vacuolation, necrosis, and inflammation infiltration while in the intestine, mucosal fold, cellular infiltration, and thickness of the muscular layers [33].

Tissues of rabbit liver and intestine were histologically examined based on the scoring system of histological alterations adapted from previous studies [25, 34]. The liver was examined for congestion, hemorrhage cytoplasmic vacuolation, hepatocyte necrosis, and features of inflammation. On the other hand, intestinal tissue was assessed regarding parameters like destroyed mucosal structure, infiltration of cells, and increased thickness of the muscular layer [33].

2.4 Carcass trait

The rabbits were slaughtered in twice during the experiment. Slaughtering was gently according to the Islamic method. Four rabbits were selected at random from each group and fasted for 12 h before being euthanized on the 5th day after infection. Furthermore, four rabbits from each group were slaughtered at the end of the feeding trial. Before slaughtered, each rabbit's live body weight was recorded. Following complete exsanguination, various body parts such as the front and back legs, head, hairless carcass, intestines, liver, lungs, kidneys, rear parts, shoulders, and ribs were weighed and meticulously documented using the methodology [35].

2.5 Statistical analysis

ANOVA was performed using a Completely Randomized Design (CRD) with an unequal number of replications as described by [36]. Duncan's multiple range test [37] was then used to assess the significance of mean performance across all treatments in each characteristic. The carcass trial data gathered during the experiment were analyzed using SAS software (2013), and means were compared using multiple tests [37]. The level of significance was set at ($p < 0.05$).

3 Results:

3.1 Characterization of nano-emulsion

3.1.1 Zeta potential

Following analysis, Fig. 1 depicts a visual representation of the zeta potential. The study discovered that the

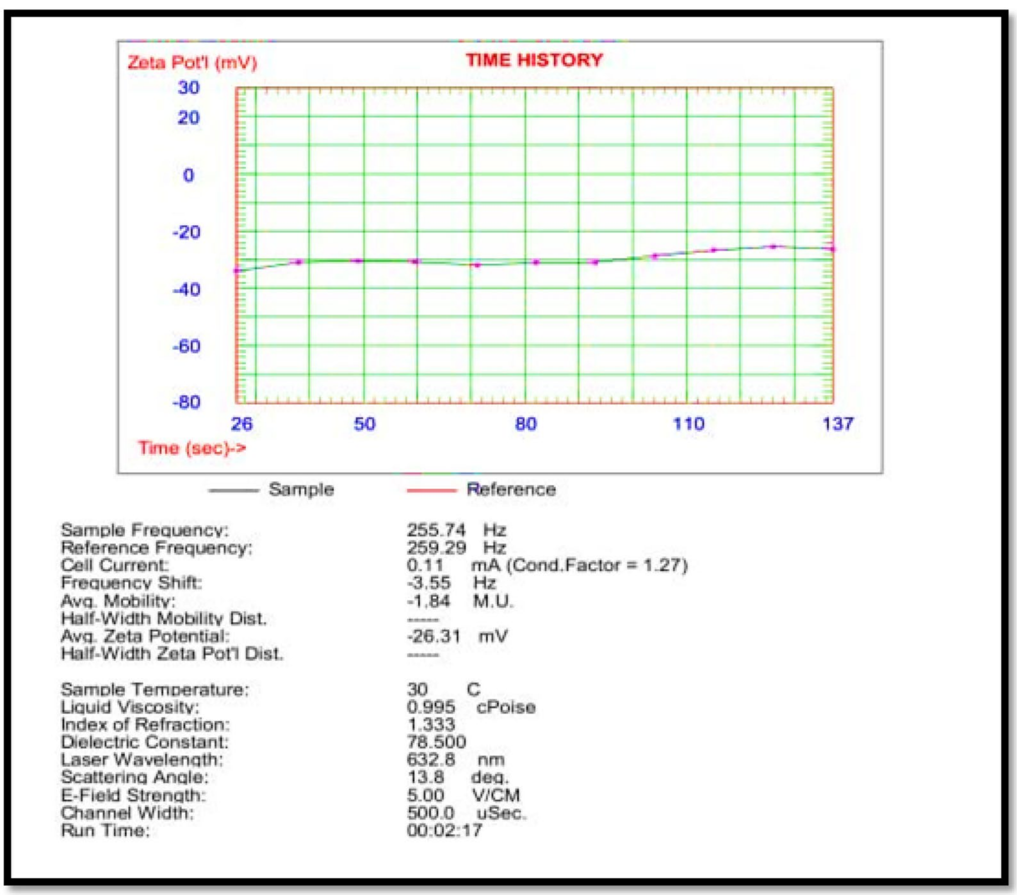

Fig. 1 Zeta Potential of Nano-Emulsified Cumin Essential Oil. The zeta potential of nano-emulsified cumin essential oil was evaluated to determine the stability of the nano-emulsion. A high absolute zeta potential indicates that the colloidal dispersion is more stable as a result of electrostatic repulsion between particles

colloidal system was relatively stable, with an average zeta potential of −26.31 mV. Furthermore, the average mobility of −1.84 M.U. suggests that the particles are negatively charged and migrate toward the anode in an electric field.

3.1.2 Transmission electron microscopy

Using TEM (FEI-TECNAI G2-20 TWIN, Netherlands), the nano-emulsion of cumin oil was measured to be 35.56, 35.90, 51.76, and 52.58 nm. Figure 2 depicts a positive representation of the nano-emulsion, showing its spherical shape, homogenous dimensions, and lack of clustering.

3.1.3 In vitro study results

The study found that the control tube, which contained only the bacterial culture, was muddy, indicating significant bacterial growth. In contrast, the broth without bacterial inoculation remained clear. Notably, the tubes containing varying quantities of nano-emulsified cumin oil produced clear, transparent soup, indicating that bacterial growth was successfully inhibited, as shown in Fig. 3. Furthermore, the blood agar plate assays gave additional proof for the nano-emulsified cumin oil's antibacterial properties. *Clostridium perfringens* in the control samples showed a clear twofold zone of hemolysis, which indicates vigorous bacterial growth. However, adding nano-emulsified cumin oil to the bacterial cultures completely inhibited bacterial growth on blood agar plates. The plates treated with the oil showed no evidence of the double zone of hemolysis, demonstrating the oil's significant inhibitory impact, as illustrated in Fig. 4.

3.2 In vivo study results

3.2.1 Clinical findings

Table 3 and Fig. 5 summarize the study's clinical observations. Infection with Clostridium perfringens type A resulted in significant clinical symptoms in rabbits, with an 82% morbidity rate and a 30% mortality rate in the untreated infected group (G3). The symptoms were anorexia, melancholy, severe diarrhea, and bloating. However, prophylactic treatment of Nano-emulsified cumin oil (G4) reduced morbidity by 25% and mortality by 5%. Furthermore, post-infection treatment (G5) improved results by lowering morbidity to 67% and mortality to 10%.

3.2.2 Postmortem findings

As shown in Table 4 and Fig. 6, postmortem examinations indicated extensive pathological abnormalities in the untreated infected group (G3), including enteritis, typhlitis, necrosis, and bleeding. In contrast, the preventative group (G4) and the therapeutic group (G5) that received nano-emulsified cumin oil had lesser lesions.

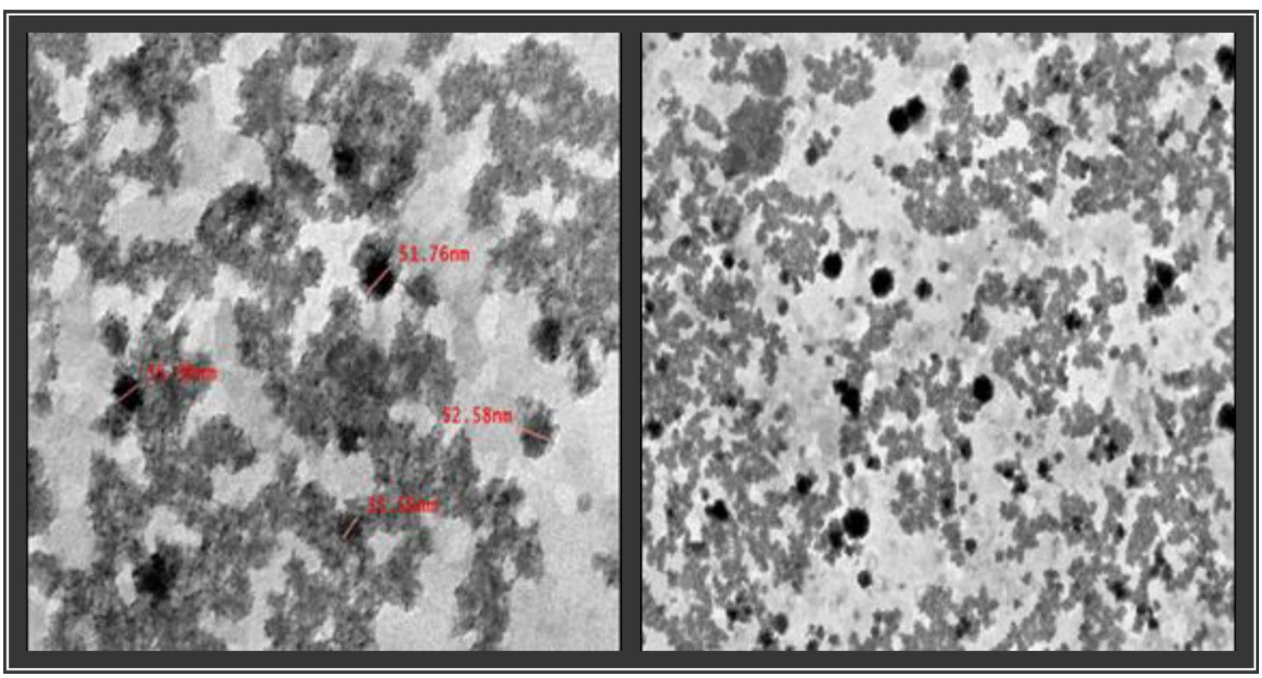

Fig. 2 Transmission Electron Microscopic Images of Nano-Emulsified Cumin Essential Oil. Transmission electron microscopy photos of nano-emulsified cumin essential oil, demonstrating particle shape and size distribution. The photographs show the successful production of nanoscale particles

Fig. 3 Growth and Inhibition of *C. perfringens* Type A on Blood Agar Plates. (**A**, **B**) The growth of C. perfringens Type A is demonstrated by the presence of a double zone of haemolysis on blood agar plates, indicating the bacterium's typical haemolytic activity. (**C**) The inhibition effect of nano-emulsified cumin essential oil on C. perfringens Type A is shown, where the absence of the double zone of haemolysis indicates effective suppression of bacterial growth

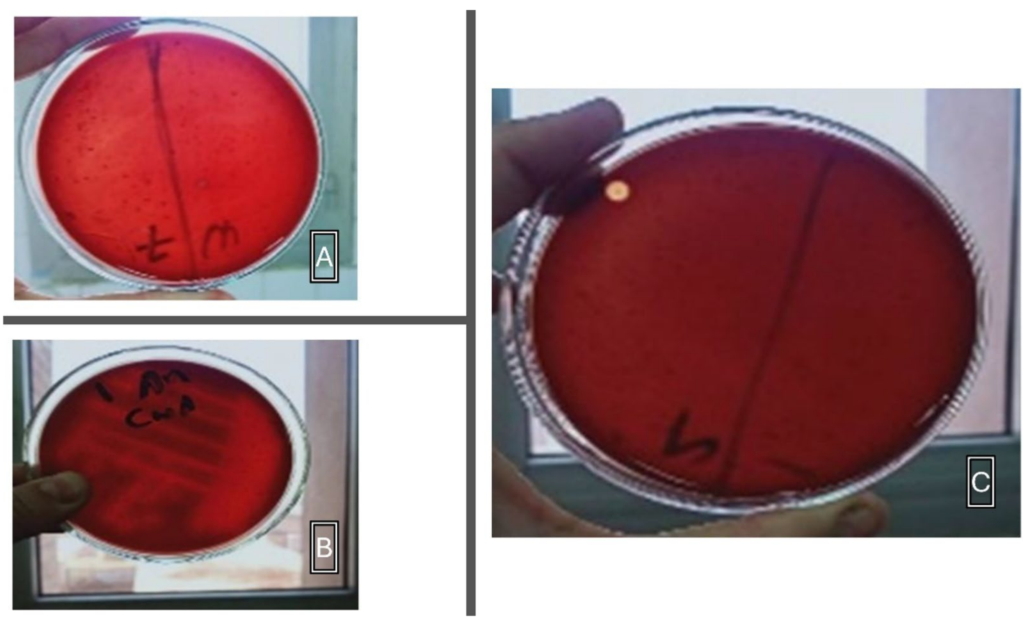

Fig. 4 Clinical Symptoms in Affected Rabbits. The affected rabbits had ruffled fur, significant bloating, and doughy dark diarrhea. These symptoms developed after being exposed to an experimental infection

Notably, the preventive group (G4) had minor pathological alterations, indicating significant protective effects, whereas the treatment group (G5) showed symptoms of tissue recovery and decreased severity of lesions. These findings give compelling evidence of nano-emulsified cumin oil's significant protective and therapeutic effects

Table 3 Protective effect of nano-emulsified cumin oil on clinical signs of *Clostridium perfringens* infection in Gabaly Rabbits

Group	Morbidity	Mortality	Depression	Tympany	Diarrhea
G1	–	–	–	–	–
G2	–	–	–	–	–
G3	82%	30%	+++	+++	+++
G4	25%	5%	+	+	+
G5	67%	10%	++	++	++

G1: (control group) received no NECO or infection. G2: (NECO control group) got NECO but no infection. G3: (positive control group) infected, received no NECO. G4: (NECO's preventive group) received NECO before infection. G5 (NECO's curative group) received NECO after infection. +: mild; ++: moderate; +++: severe

in combating Clostridium perfringens infection, particularly when taken prophylactically.

3.2.3 Histopathological findings

The histological study, as shown in Fig. 7, and the lesion score in Table 5, revealed that nano-cumin oil emulsion had considerable preventive and therapeutic benefits against Clostridium-induced damage in both liver and intestinal tissues. In the liver tissue, the control group showed well-structured hepatic lobules, central vein, and hepatic sinusoids and nano-cumin oil emulsion groups showed little degenerative changes; cytoplasmic vacuolation in hepatocytes was observed in focal areas, whereas the Clostridium-infected group showed significant harm, including vascular congestion, lymphocytic aggregations, and hepatocyte necrosis. The administration of nano-cumin oil emulsion, both preventively and therapeutically, resulted in near-normal tissue morphology with lower harm ratings, mild vascular congestion, and vacuolation of hepatocytes.

In the intestine, both the control and nano-cumin oil emulsion groups maintained normal villi structure. In contrast, the Clostridium-infected group showed severe degeneration and necrosis of the intestinal villi with significant lymphatic cell aggregations. Preventive administration of nano-cumin oil emulsion caused protective hyperplasia and minor desquamation, but the treatment group showed evidence of epithelial regeneration and normal tissue architecture, with some edema.

3.2.4 Growth performance and carcass traits of Gabaly growing rabbits

In terms of growth performance, as shown in Table 6 and Fig. 8, the group supplemented with nano-emulsified cumin oil (G2) had the best results, with the highest final body weight (2.32 kg), average daily gain (20.02 g/day), and feed conversion ratio (3.80). In contrast, the infected group without supplements (G3) had the poorest results, with the lowest final body

Fig. 5 Detailed Symptoms in Affected Rabbits. The affected rabbits showed ruffled fur, severe bloat, and diarrhea, with the stool sometimes tinged with blood. This figure provides a closer examination of the clinical symptoms observed

Table 4 Effect of nano-emulsified cumin oil on post-mortem lesion scores

Groups	Lesion scores	
	Liver	Cecum
G1	0^{c}	0^{c}
G2	0^{c}	0^{c}
G3	2.33 ± 0.33^{a}	3 ± 0.21^{a}
G4	0.66 ± 0.33^{c}	0.66 ± 0.58^{c}
G5	1.6 ± 0.33^{ab}	$1.6b \pm 0.41^{b}$

G1: (control group) received no NECO or infection. G2: (NECO control group) got NECO but no infection. G3: (positive control group) infected, received no NECO. G4: (NECO's preventive group) received NECO before infection. G5 (NECO's curative group) received NECO after infection

weight (1.87 kg), average daily increase (14.76 g/day), and the least efficient feed conversion ratio (5.31). Both the pre-infection (G4) and post-infection (G5) groups that received nano-emulsified cumin oil supplementation outperformed the control group (G1) and the infected group, with the pre-infection supplementation group doing somewhat better. Carcass features were tested five days after infection and are shown in Table 7, suggesting considerable benefits of NECO supplementation. The pre-infection group that received NECO supplementation (G4) had the highest carcass weight (0.43 kg), as well as significant results for fur (103.25 g), intestine weight (158.47 g), and round weight (156.03 g). In contrast, the infected group (G3) had the lowest values, showing a detrimental impact of the infection.

4 Discussion

One of the most significant challenges in the rabbit sector is infectious intestinal infections, especially those caused by *Clostridial* species, which cause bloating as well as elevated mortality rates during the period of weaning [38]. Antibiotics have traditionally been used to control these infections; however, this strategy has drawbacks such as antimicrobial resistance and potential rabbit toxicity. As a result, there is a growing interest in discovering natural alternatives with antibacterial qualities, such as spices [17]. The scientific reports published in the recent past have shown that spices possess antibacterial substance, encouraging the intention for more advanced research in this segment of common use (Silva and Domingues, 2017) (Table 8).

Here in the current study, we determined the effectiveness of NECO as an antibacterial and growth promoter. We also evaluated its effectiveness in vitro for newly weaned Gabaly rabbits where sprayed pelleted ration was used as the preventive as well as the therapeutic treatment of *C. perfringens* type A infection. In this in vitro study, NECO was tested at four different concentrations that included 0. 1, 0. 2, 0. 5, and 1 ml in both the media broth and blood agar. Hence, the research findings substantiated that NECO efficiently suppressed bacterial growth in all oil concentrations. Agar diffusion and dilution methods were used to analyze antimicrobial effects of cumin oil, against the Gram-positive and the Gram-negative bacterial strains such as *E. coli, S. aureus, S. faecalis, P. aeruginosa, and K. pneumonia* [39].

Based on these promising findings, we investigated the impact of NECO on the response of weaning Gabaly rabbits to C. perfringens type A. The challenge produced substantial clinic-pathological results in the

Fig. 6 Pathological Changes in the Liver and Intestines of Rabbits (**A**) The liver displayed severe congestion, enlargement, sub-capsular hemorrhage, and necrosis. (**B**) The large intestine exhibited ballooning and gas accumulation, with odema and petechial hemorrhages. (**C**) The small intestine presented different degrees of enteritis and engorged mesenteric blood vessels

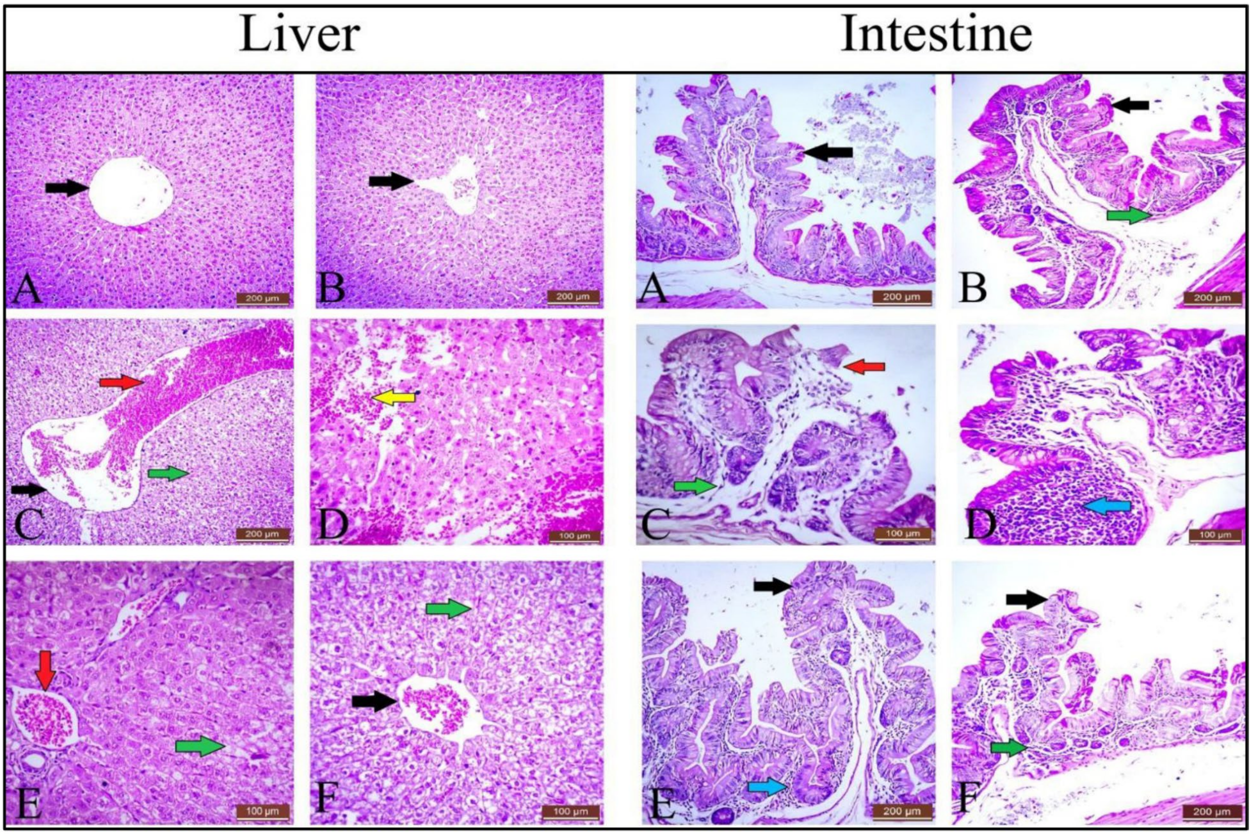

Fig. 7 photomicrographs of Liver and Intestine of Rabbits in Different Experimental Groups Photomicrographs of liver and intestinal sections in different experimental groups stained with H&E. Liver sections: (**A**) Control group with normal hepatic architecture. (**B**) Nano cumin oil emulsion group with mostly normal hepatic features. (**C**) Clostridium-challenged group showing vascular congestion, perivascular oedema, and hepatocyte vacuolation. (**D**) Clostridium-challenged group with necrosis and lymphocytic aggregations. (**E**) Clostridium-challenged with preventive nano cumin oil emulsion group showing mild congestion and vacuolation. (**F**) Clostridium-challenged with treatment nano cumin oil group with moderate degenerative alterations. Key: (Black arrow) central vein; (Red arrow) congested vessels; (Yellow arrow) hemorrhagic necrotic area; (Green arrow) hepatocyte vacuolation. Intestinal sections: (**A**) The control group has conventional villous architecture. (**B**) The nano cumin oil emulsion group has normal villi. (**C** and **D**) The Clostridium-challenged group exhibited villous degradation, epithelium loss, and inflammatory cell aggregations. (**E**) Clostridium-challenged group treated with preventative nano cumin oil emulsion exhibits moderate epithelial hyperplasia and superficial desquamation. (F) Clostridium-challenged group treated with nano cumin oil demonstrates regenerating epithelial characteristics and oedema. Key: Intestinal villi (black arrows); necrotic sloughed epithelium (red arrows); lamina propria (green arrows); and inflammatory cell aggregations (blue arrows)

Table 5 Hepatic and intestinal injury scores in Gabaly rabbits

Group	Cumulative liver injury scores	Cumulative intestinal injury scores
G1	0.08	0.12
G2	0.2	0.12
G3	1.12	1.16
G4	0.48	0.56
G5	0.6	0.72

G1: (control group) received no NECO or infection. G2: (NECO control group) got NECO but no infection. G3: (positive control group) infected, received no NECO. G4: (NECO's preventive group) received NECO before infection. G5 (NECO's curative group) received NECO after infection

control positive group (G3), such as anorexia, depression, ruffled fur, severe diarrhea with a foul smell and occasional bloodstains on the perineum and hindquarters, significant bloat, and varying degrees of mortality. The postmortem examination showed severe congestion, enlargement, subcapsular bleeding, and necrosis in the liver. The rabbits' big intestines ballooned and accumulated gas, whereas the caecal serosa displayed edema with petechial or ecchymotic bleeding. The caecum and colon had foul-smelling, watery, dark brown contents filled with gasses. These findings are identical with those reported by [40] in spontaneously infected rabbits with Clostridial pathogens. While NECO showed significant protection against Clostridium

Table 6 Growth performance of Gabaly rabbits fed different diets

Groups	Initial BW (kg)	FBW (kg)	Daily DMI (g/h/d)	Total weight gain (g)	ADG (g)	FCR
G1	0.56 ± 0.13	2.03^{bc} ± 0.1	78	1474.4^{b} ± 151.8	16.38^{b} ± 1.7	4.8^{b} ± 0.5
G2	0.54 ± 0.11	2.32^{a} ± 0.07	76	1801.76^{a} ± 107.62	20.02^{a} ± 1.2	3.8^{d} ± 0.2
G3	0.54 ± 0.11	1.88^{d} ± 0.09	78	1328.7^{c} ± 108.7	14.76^{c} ± 1.2	5.3^{a} ± 0.4
G4	0.56 ± 0.12	2.08^{b} ± 0.1	76	1535.8^{b} ± 177.7	17.06^{b} ± 2	4.5^{c} ± 0.5
G5	0.53 ± 0.09	2^{c} ± 0.07	78	1483.1^{b} ± 135.1	16.47^{b} ± 1.5	$4.8b^{c}$ ± 0.4
LSD 5%	N.S	0.063	–	89.81	0.99	0.27
significant	N.S	**	–	**	**	**

Differences superscript letter are significantly differed ($P < 0.05$). G1: (control group) received no NECO or infection. G2: (NECO control group) got NECO but no infection. G3: (positive control group) infected, received no NECO. G4: (NECO's preventive group) received NECO before infection. G5 (NECO's curative group) received NECO after infection. Initial body weight (BW), final body weight (FBW), daily dry matter intake (DMI), total weight gain, average daily gain (ADG), and feed conversion ratio (FCR)

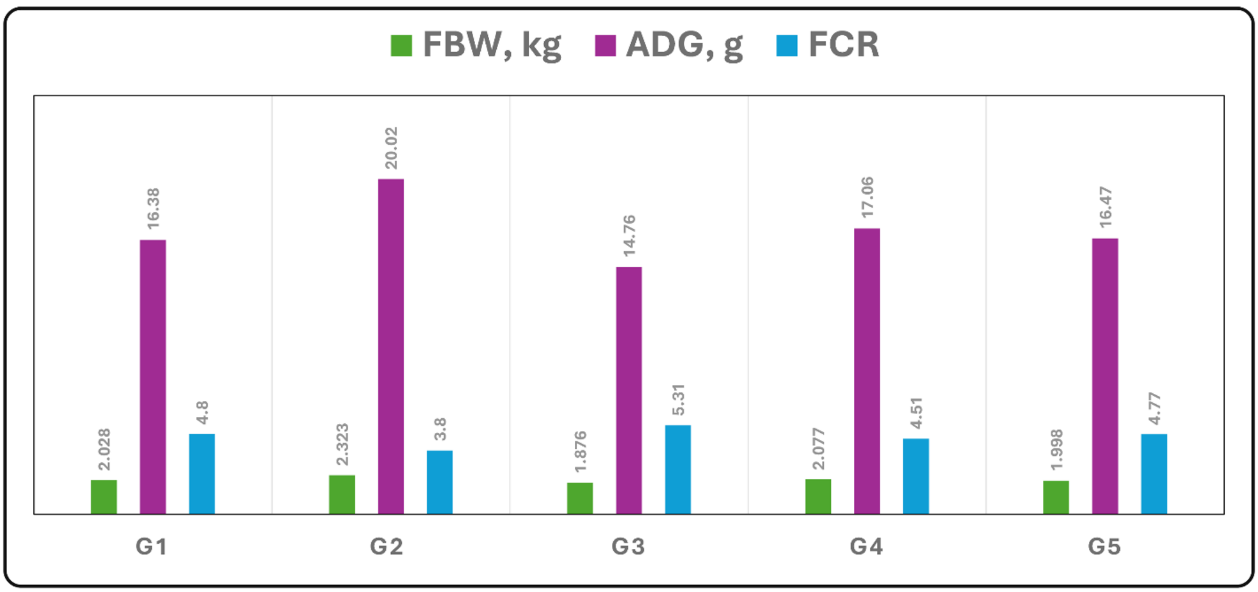

Fig. 8 Effect of Dietary Nano-Emulsified Cumin Essential Oil on Growth Performance of Growing Rabbits. The growth performance of growing rabbits fed with nano-emulsified cumin essential oil. Parameters such as weight gain and feed conversion ratio are presented to assess the impact of the dietary intervention

perfringens infection, both preventive (G4) and curative (G5) NECO-treated groups had significantly lower morbidity, severe clinical manifestations, postmortem findings, and mortality rates than the infected control group (G3). This is reinforced by the studies which discovered that essential oils can reduce pathogen levels in the stomach, including gram-negative E. coli and Salmonella [41, 42]. These essential oils have also been found to exhibit antibacterial properties against Salmonella, Campylobacter, Clostridium jejuni, E. coli O157:H7, and Listeria monocytogenes in a variety of non-poultry food matrices. Our findings are consistent with [43], who addressed cumin's traditional medicinal uses in treating digestive issues and increasing liver function.

These findings can be explained further by [44, 45], who highlighted the favorable impacts of photobiotic feed additives (PFA) due to their antibacterial and antioxidant capabilities. Dietary PFA can change and balance intestinal bacteria, slow down the formation of toxic substances of microbes, and eliminate intestinal troubles and immunological pressure because of its bacteriostatic effects on multiple pathogenic bacteria. In broiler production, curative effects of essential oils in different conditions have been proved by the augmentation of digestive enzyme activity, decrease in products of fermentation and

Table 7 Effect of different experimental diets on rabbit carcasses after five days from infection

group	LBW (Kg)	Carcass (Kg)	Fur (g)	Legs (g)	Hand (g)	Heart (g)	Intestines (g)	Lung (g)	Liver (g)	Kidney (g)	Shoulders (g)	Lion (g)	Round (g)
G1	0.95^{a}	0.30^{d}	73.20^{b}	19.83^{b}	8.57^{ab}	2.42^{c}	131.77^{c}	6.10	25.07^{c}	7.50^{c}	82.37^{b}	53.57^{e}	101.70^{d}
G2	0.89^{b}	0.41^{b}	91.71^{a}	23.33^{ab}	9.99^{b}	3.20^{b}	152.00^{b}	5.30	35.13^{b}	9.40^{b}	109.17^{a}	104.58^{b}	144.69^{b}
G3	0.86^{bc}	0.37^{c}	88.33^{a}	22.85^{ab}	9.13^{ab}	2.27^{c}	150.52^{b}	5.48	43.43^{a}	9.80^{ab}	93.87^{ab}	91.85^{d}	128.87^{c}
G4	0.84^{c}	0.43^{a}	103.25^{a}	23.17^{ab}	9.55^{ab}	3.27^{b}	158.47^{a}	4.93	45.77^{a}	10.60^{ab}	104.23^{ab}	113.32^{a}	156.03^{a}
G5	0.68^{d}	0.38^{c}	94.75^{a}	25.25^{a}	11.40^{a}	4.25^{a}	140.00^{c}	5.95	49.05^{a}	11.20^{a}	85.00^{ab}	97.50^{c}	132.50^{c}
S.E	±0.01	±0.01	±4.61	±1.20	±0.70	±0.19	±0.94	±0.38	±2.25	±0.47	±7.67	±1.15	±1.48
Sig	***	***	*	*	*	*	***	NS	**	*	*	**	***

Differences superscript letter is (Sig.) significantly differed ($P < 0.05$). G1: (control group) received no NECO or infection. G2: (NECO control group) got NECO but no infection. G3: (positive control group) infected, received no NECO. G4: (NECO's preventive group) received NECO before infection. G5 (NECO's curative group) received NECO after infection

Table 8 Final carcass traits of Gabaly rabbits at experiment conclusion

group	LBW (Kg)	Carcass (Kg)	Fur (g)	Legs (g)	Hand (g)	Heart (g)	Intestines (g)	Lung (g)	Liver (g)	Kidney (g)	Shoulders (g)	Lion (g)	Round (g)
G1	1.82^{c}	1.09^{b}	292.50^{b}	40.75^{ab}	17.22^{ab}	8.37^{a}	171.25^{c}	8.4^{b}	55.84^{a}	17.56^{a}	257.50^{a}	322.75^{d}	388.25^{a}
G2	1.99^{a}	1.25^{a}	345^{a}	45.4^{a}	18.95^{a}	8.62^{a}	227^{a}	13.35^{a}	58.07^{a}	18.65^{a}	241.25^{b}	373.75^{a}	361^{ab}
G3	1.36^{e}	0.76^{d}	235^{c}	34.13^{b}	14.59^{b}	5.5^{b}	175.25^{c}	7.44^{b}	40.17^{b}	13.63^{b}	167.5^{e}	247.5^{e}	277.5^{c}
G4	1.87^{b}	0.94^{c}	333.75^{a}	41.92^{a}	19.44^{a}	8.24^{a}	233.75^{a}	8.79^{b}	49.41^{a}	18.7^{a}	227^{c}	344.75^{b}	362.25^{ab}
G5	1.77^{d}	0.94^{c}	293.75^{b}	40.81^{ab}	19.72^{a}	5.03^{b}	190.25^{b}	9.19^{b}	49.66^{a}	16.8^{ab}	213.75^{d}	331^{c}	344.25^{b}
S.E	±0.01	±0.01	±4.31	±2.19	±1.19	±0.42	±2.88	±0.68	±2.91	±1.21	±1.94	±2.70	±10.86
Sig	***	***	***	*	*	***	***	***	**	*	***	***	***

Differences superscript letter is (Sig.) significantly differed ($P < 0.05$). G1: (control group) received no NECO or infection. G2: (NECO control group) got NECO but no infection. G3: (positive control group) infected, received no NECO. G4: (NECO's preventive group) received NECO before infection. G5 (NECO's curative group) received NECO after infection pathogens, improved nutrient digestion, and increased bioavailability of essential nutrients [46].

These findings can be further explained by those who stressed the antibacterial and antioxidant activities of PFA. The consumption of dietary PFA can influence and restore the balance of the intestinal bacteria, restrict bacterial generation of toxic matters, and help to overcome intestinal troubles and immunological pressure through its direct antibacterial effect on assorted pathogenic bacteria. In broiler production, due to its ability to increase the activity of digestive enzymes, decrease production of fermentation products and pathogens, enhance nutrient digestion and availability of important nutrients, essential oils provide good therapeutic benefits in many instances. Liver and intestinal histopathological examination is vital in assessing the general health of the body and the presence of any histopathological changes. Histological changes caused by Clostridium infection have been observed to be serious and include hepatic vascular congestion, necrosis, and lymphocyte aggregation to prove inflammation and tissue damage. In a similar manner, this applies to degeneration, necrosis as well as hyperplasia in the gut. These findings are supporting with the findings of previous studies regarding the pathogenic properties of Clostridium species [9, 47]. The lesion, liver, and intestinal injury scores were lower in the treated groups postmortem, and therefore, this research suggests that NECO had protective roles. This goes to show that with the use of NECO, the infection rate of Clostridium perfringens can be effectively managed. The maintenance of the organ integrity thus enhances general health and growth due to enhanced nutrient uptake from the body and metabolism. The growth performance indices such as final body weight, weight gain, and feed conversion ratio were higher in the NECO-treated groups, comparing the results of these groups, G2 which is the NECO control group had higher results than the untreated control group G1. In the following post-infection also, the preventive NECO group (G4) was found to be significantly superior to the positive control group (G3) which supports the fact that NECO has potential to minimize the impacts of infection on growth. Also, the result of the carcass features demonstrated that groups G2 and G4 had significant increase in live body, carcass and organ weight. This prove that NECO improves animal growth performance and meat quality. These finding are consistent with the favorable effects reported for essential oils.

It is possible to connect these positive outcomes of NECO with numerous processes. Among these methods, one is the antibacterial action that is considered to have crucial significance for reducing the number of bacteria as well as inhibiting the multiplication of Clostridium perfringens. Cumin oil has anti-inflammatory properties which can lessen the intensity of clinical signs and histopathological changes resulting from infection. Another possible mode through which NECO acts is through enhancement of gut health and its function to increase nutrients and thus support growth and health. These methods are supported by studies on the different essential oils and nano-emulsion that have been discovered, all of which has similar benefits [48].

The anti-diarrheal property of cumin seeds is known traditionally. In experimental study on albino rats which have diarrhea induced by castor oil, the aqueous extract of cumin seeds reduced the frequency of diarrhea, delayed the time for defecation, decreased intestine fluid output, and inhibited intestinal peristalsis in a dose dependent manner at doses of 100, 250, and 500 mg/kg [47]. Cumin essential oil has ability to enhance animal's growth performance. It contains bioactive chemicals like cumin aldehyde which has anti-microbial activity. These compounds also enhance a healthy gut microbial flora by suppressing the undesirable bacteria, thereby enhancing nutrient digestion and health in the general management of animal production [46]. Furthermore, cumin essential oil is also a source of antioxidants that decrease the level of oxidized materials in animals. Reduction in the level of oxidative stress enhances immunity and general well-being resulting in better growth rates [49].

Studies have shown that cumin essential oil increases the activity of the enzymes in the digestive tracts thereby improving digestion and absorption of nutrients leading to improved growth rates in animals [50]. Moreover, the cumin essential oil has anti-inflammatory effects that help the body to decrease inflammation within the gut which in turn enhances the digestion and absorption of nutrients [35]. Previous research has shown that incorporation of free-form cumin essential oil supplement in broiler diets has positive outcomes [51]. Adding 0.2% of ground cumin to the diet has been reported to improve the FCR in broilers [52]. Cumin's bioactive compounds might enhance the secretion of digestive enzymes due to the enhance of bile secretion and pancreatic secretions [51]. It is worth to note that enhancing the growth performance of broiler chicken through the delivery of cumin essential oil through nano-encapsulation is more efficient compared to antibiotic treatment due to the application of chitosan nanoparticles for the formation of the capsule in delivering cumin essential oil has been proved to have antibacterial activity which enhances the growth performance of the broiler chicken as stated by [53].

Some studies have established that cumin oil is effective in animal feed. For instance, broilers fed on cumin oil had significantly higher weight gains, feed conversion ratios, and general health coefficients over the broilers fed on the non-cumin oil diets [54]. In the same respect, dairy cows treated with cumin oil had better milk production and quality, along with better indicators of gut health [55].

Recent research has provided deeper insights into the antibacterial properties of nano-emulsified cumin oil (NECO) and its influence on rabbit health and growth performance. Study explored the formulation and characterization of cumin essential oil nano-emulsion (CEONE), assessing its role as both an antibacterial agent and a growth enhancer in broiler chickens. Their results indicated that CEONE supplementation contributed to increased weight gain and a favorable microbial balance, highlighting its potential as a multifunctional additive in poultry farming [56].

Likewise, study during 2024 examined the impact of nano-emulsified vegetable oils on growth performance, hematological and biochemical parameters, oxidative stress, and gut microbiota in rabbits. Their study demonstrated notable improvements in final body weight, feed conversion efficiency, and carcass characteristics in New Zealand White and V-line rabbits [57]. Additionally, they observed elevated serum protein and globulin levels, alongside reductions in total lipid and cholesterol concentrations. The research also revealed a decline in total bacterial counts, as well as a decrease in *Escherichia coli* and *lactobacilli populations* in the jejunum and colon, indicating enhanced intestinal health. NECO is a convenient and effective means for enhancing rabbit health status, decreasing the level of infections and enhancing growth and carcass characteristics.

5 Conclusion

This study demonstrated that nano-emulsified cumin oil (NECO) exhibits strong antibacterial activity against *Clostridium perfringens* type A, effectively inhibiting bacterial growth in vitro. In vivo trials further confirmed that dietary supplementation with NECO, particularly at a dosage of 10 mL/kg diet, significantly reduced morbidity and mortality rates in infected rabbits. Administering NECO before infection mitigated clinical symptoms, postmortem lesions, and histopathological alterations associated with *C. perfringens* infection. Additionally, NECO supplementation improved growth performance, feed conversion efficiency, and overall health in rabbits. Given its enhanced bioavailability and stability, NECO presents a promising natural alternative for preventing and managing *Clostridial* infections in rabbit farming. These findings suggest that NECO can be effectively integrated into animal feed as both a prophylactic and therapeutic agent, offering a sustainable strategy to enhance livestock productivity while reducing reliance on conventional antibiotics. More research should be done to enhance the stability of NECO and ascertain more features about its mode of action and whether it can be utilized in other breeds of livestock. Furthermore, its long-term implications on profitability and risks associated with incorporating NECO into commercial farming processes would be valuable.

6 Ethics approval and consent to participate

The study protocols were approved by the Institutional Animals Care and Use Committee, Research Ethics (No.: BUFVTM 08-03-23) following animal welfare guidelines. All efforts were made to minimize animal suffering and to use only the number of animals necessary to achieve reliable scientific data.

Abbreviations

ESI	Emulsion stability index
NECO	Nano-emulsified cumin essential oil
NEs	Nano-emulsions
PFA	Photobiotic feed additives
TEM	Transmission electron microscopy

Author's contribution

RRF, MA, ZSM, and SHM contributed to the conceptualization, methodology, and investigation of the study, with RRF, ZSM, and SHM also drafting the original manuscript MA further contributed to data curation, formal analysis, and the critical review and editing of the manuscript AHT conducted the histopathological examination, while MA and NMK provided substantial input through bacterial culture preparation, manuscript writing, and critical revisions All authors reviewed and approved the final version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Conflict of interest

The authors declare no competing interests.

Consent for publication

Not applicable.

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