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Dietary essential oil and protected sodium butyrate modulate digestive organ traits, intestinal morphology, and mortality in broiler chickens

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Abstract. Essential oil (EO) and protected sodium butyrate (PSB) are potential alternatives to antibiotics in broiler diets. This study compared the effects of EO and a combination of EO and PSB on growth performance, carcass traits, digestive organ characteristics, intestinal morphology, and mortality in broiler chickens. A total of 2 400 one-day-old male broilers were randomly assigned to four dietary treatments: a control diet (CO), 0.1 g/kg enramycin (AB), 1 g/kg EO, and 0.25 g/kg EO + 0.75 g/kg PSB (ES), each with six replicates. One bird per pen was selected on days 21 and 35 to evaluate carcass traits and digestive tract length and weight. Growth performance variables and carcass traits did not differ between treatments ($P > 0.05$). However, mortality was higher in the AB group at several time points ($P < 0.05$). The ES treatment increased crop, gizzard, and liver weights on day 21 ($P < 0.05$) and gizzard weight on day 35, while EO reduced caecum weight on day 35. ES also increased the lengths of the small intestine and jejunum on day 21 ($P < 0.05$). On day 21, AB, EO, and ES increased colon length compared to CO ($P < 0.05$). Jejunal morphology showed reduced crypt depth and a higher villus-to-crypt ratio in all treatment groups ($P < 0.05$). On day 35, ES resulted in the shortest ileal villi and the greatest villus width, whereas EO reduced villus surface area ($P < 0.05$). In conclusion, dietary EO combined with PSB altered selected digestive organ traits and intestinal morphology in broiler chickens, whereas enramycin increased mortality without improving growth performance.

Keywords: broiler chickens, digestive organ, essential oil blend, growth performance, intestinal health, protected sodium butyrate

Running title: Dietary essential oils and butyrate in broiler nutrition

Introduction

The use of antibiotics in broiler production contributes to the emergence of antibiotic-resistant bacteria, posing a serious public health problem worldwide. As awareness of these risks grows, consumers are increasingly seeking antibiotic-free broiler products due to concerns about antibiotic residues and the perception that such products are healthier and safer than conventionally produced meat (Haque et al., 2020; Zhu et al., 2021; Puvača and Tufarelli, 2023; Gyamfi et al., 2024; Kozera-Kowalska and Uglis, 2024; Sampath and Kim, 2025). In this context, broilers industry is actively exploring several alternatives to antibiotic growth promoters, including prebiotics, probiotics, essential oils (EOs), and other natural feed additives, to maintain bird health and optimal performance without relying on antibiotics (Abd El-Hack et al., 2022; Puvača and Tufarelli, 2023; Sampath and Kim, 2025).

EOs are plant-derived volatile compounds that have gained considerable attention as natural alternatives to antibiotic growth promoters in animal production systems (Lee et al., 2004; Puvača et al., 2013a; Adaszyńska-Skwirzyńska and Szczerbińska, 2017). Oregano EO, primarily derived from *Origanum vulgare*, is characterised by a complex phytochemical profile comprising more than 20 bioactive compounds, with thymol, carvacrol, p-cymene, and γ-terpinene representing its predominant constituents (Gökalp and Demir, 2019; Sachivko et al., 2023; Veljovic et al., 2023). These compounds are responsible for the antimicrobial, antioxidant, and anti-inflammatory properties of oregano EO (Nurzyńska-Wierdak and Walasek-Janusz, 2025). Ginger EO, obtained from *Zingiber officinale*, also contains numerous volatile compounds, with zingiberene, camphene, eucalyptol, neral, and geranial among its main components. These compounds contribute to its characteristic aroma and functional properties (Hirmizi et al., 2015; Thao et al., 2023; Nguyen et al., 2025).

Clove EO, derived from the dried flower buds of *Syzygium aromaticum*, is composed mainly of eugenol, which accounts for 50–90% of the oil and is responsible for most of its antioxidant, antimicrobial, and anti-inflammatory properties (Prashar et al., 2006; Haro-González et al., 2021). β-caryophyllene, eugenyl acetate, and α-humulene are also present in varying amounts, and contribute to its bioactivity (Haro-González et al., 2021; Stancu et al., 2025). Cinnamon EO, obtained from species of the genus *Cinnamomum*, contains bioactive compounds including cinnamaldehyde, eugenol, cinnamic acid, and linalool. It is widely used in medicine and agriculture and is well known for its characteristic aroma (Guo et al., 2024; Huang et al., 2025). The use of EOs in animal feed has increased as an alternative to antibiotic growth promoters, mainly due to concerns about antibiotic residues in meat and the occurrence of antibiotic-resistant microorganisms (Azevedo et al., 2016; Oliveira et al., 2024; Rocha et al., 2024).

Dietary EOs were reported to improve growth performance and feed efficiency in broilers (Gole et al., 2022; Jawale et al., 2024; Islam et al., 2025). EO supplementation can also enhance broilers productivity by increasing digestive enzyme activity (Liu et al., 2018; Youssef et al., 2021; Elbaz et al., 2022), maintaining the balance of the intestinal microbiota (Giannenas et al., 2016; Chowdhury et al., 2018; Liu et al., 2018; Alie-Laleh et al., 2022; Ghazanfari et al., 2023), improving intestinal morphology (Chowdhury et al., 2018; Youssef et al., 2021; Alie-Laleh et al., 2022; Ghazanfari et al., 2023), and supporting intestinal barrier function (Liu et al., 2019; Ibrahim et al., 2022; Olfati and Hosseini, 2022; Huang et al., 2024). EO supplementation has also been associated with improved immune responses and reduced inflammation in broilers (Liu et al., 2019; Ibrahim et al., 2022; Olfati and Hosseini, 2022; Belhadj Slimen et al., 2025). However, other studies reported that EO addition did not improve the growth performance in poultry (Riyazi et al., 2015; Wang et al., 2019; Sarac and Yoruk, 2023).

In addition to EOs, protected sodium butyrate (PSB) has been investigated for its effects on growth performance and feed efficiency in broiler production (Shao et al., 2016; Wan et al., 2022; Zhao et al., 2022; Hanim et al., 2023; Kihal et al., 2025). Dietary supplementation with PSB at 0.5 g/kg was effective in maintaining goblet cell number and exerted beneficial effects on intestinal morphology (Sadurní et al., 2022a) A dose of 1 g/kg also retained goblet cell numbers, reduced intraepithelial lymphocyte counts, and enhanced growth performance and feed conversion ratio (FCR) compared to the control (Sadurní et al., 2022a; Wan et al., 2022; Hanim et al., 2023; Melaku et al., 2024). PSB is formulated within a physical or chemical matrix to reduce its dissociation in the upper gastrointestinal tract (Ahsan et al., 2016; Zhao et al., 2022). Dietary PSB may also improve performance by affecting digestive tract morphology (Chamba et al., 2014; Shao et al., 2016; Hanim et al., 2023; Kihal et al., 2025). In addition to providing energy for intestinal epithelial cells, butyrate stimulates their proliferation, differentiation, and barrier function (Guilloteau et al., 2010). Because EO and PSB work through different mechanisms, their combined use may produce complementary effects, with EO affecting the gut microbiota and PSB supporting intestinal structure. This combination may therefore provide a more comprehensive approach to maintaining normal intestinal function in broilers.

The combination of EO and PSB may improve broiler performance compared with their individual use; however, previous findings have been inconsistent and often dependent on the experimental conditions, particularly pathogen challenge. According to Jerzsele et al. (2012), dietary supplementation with 1.5 g/kg of an EO–PSB combination improved intestinal morphology and broiler performance compared to both the control group and the individual additives. Although dietary EO (0.1 g/kg) + PSB (1 g/kg) did not affect growth indices, it significantly reduced *Salmonella* colonisation (Cerisuelo et al., 2014). Similarly, supplementation with 0.5 g/kg of an EO blend combined with PSB at 0.5 g/kg, modified several metabolic pathways associated with the normal functional activity of the caecal microbiota and reduced the negative effects of necrotic enteritis (Bortoluzzi et al., 2018). Broilers supplemented with an EO blend in drinking water and sodium butyrate (SB) in the feed showed improved nutrient digestibility of inorganic matter, longer villi, and increased proliferation of *Lactobacillus* spp. (Katcha et al., 2021).

The synergistic interaction between SB and EO has been reported to influence gut health and growth performance in broilers, suggesting their potential as alternatives to antibiotic growth promoters. However, information comparing the effects of EO alone, EO combined with PSB, and antibiotic supplementation under non-challenged, commercial-like conditions is limited. Therefore, this study evaluated the effects of dietary EO and its combination with PSB in comparison to an antibiotic treatment, on growth performance, digestive organ development, intestinal morphology, and carcass characteristics of broilers at different production stages. It was hypothesised that the combined supplementation of EO and PSB would have a greater effect on gastrointestinal morphology and related production parameters compared to EO alone or antibiotic supplementation.

Material and methods

The experiment was conducted at the closed-house research facility of the Faculty of Animal Science, Universitas Gadjah Mada, Indonesia. The experimental protocol was approved by the Ethical Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia (ID: 0009/EC-FKH/Eks./2021).

Birds, diets and housing

A total of 2 400 one-day-old male New Lohmann broilers (40–45 g) were used in a completely randomised feeding trial with four dietary treatments. All birds were vaccinated at the hatchery according to standard commercial practices prior to placement. Birds were randomly assigned to one of four dietary treatments, each with six replicate pens (100 birds per pen; pen size: 5 m × 2 m). The treatments consisted of a basal diet (CO), a basal diet supplemented with 0.1 g/kg of the antibiotic enramycin (AB), a basal diet supplemented with 1 g/kg of an EO blend (oregano, cinnamon, clove, ginger , and other EOs; EO-FIT® Poultry, Norel S.A., Madrid, Spain) (EO), and a basal diet supplemented with 0.25 g/kg of the EO blend plus 0.75 g/kg of PSB (sodium butyrate protected with sodium salts of palm fatty acid distillates; 70% sodium butyrate equivalent to 56% butyric acid; GUSTOR N'RGY®, Norel S.A., Madrid, Spain) (ES). The basal diet, based on maize and soybean meal, was formulated to provide 2 960, 3 073, and 3 183 kcal/kg apparent metabolizable energy and 22.79, 21.37, and 19.32% crude protein during the starter (days 0–10), grower (days 11–21), and finisher (days 22–35) phases, respectively (Table 1). Feed and water were provided *ad libitum* throughout the experiment. Birds were reared in floor pens bedded with rice husk litter within an environmentally controlled closed-house system. The brooding temperature was initially set at 33 °C and gradually reduced to 25 °C by the end of the rearing period. Lighting and environmental conditions were maintained according to the recommendations of the New

Lohmann Broiler Management Guide throughout the experimental period. Animal care and experimental procedures also complied with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010).

Growth performance

Feed intake (FI) and body weight (BW) were recorded weekly throughout the experimental period. Body weight gain (BWG) was calculated as the difference between final and initial body weight, with measurements recorded on a pen basis. FCR was calculated as the ratio of feed intake (FI) to BWG and corrected for mortality. Mortality was recorded daily as the percentage of birds that died relative to the initial number of birds in each pen. The performance index (PI) was calculated following Aprianto et al. (2023):

$$\mathrm{PI} = \frac{\text{livability (\%)} \times \text{body weight (kg)}}{\text{feed conversion ratio} \times \text{age (days)}}.$$

Carcass trait and internal organ measurements

On days 21 and 35 of the feeding trial, one broiler chicken with a BW close to the mean BW of its pen was selected from each replicate, resulting in a total of 24 birds per sampling day (4 treatments × 6 replicates). Birds were fasted for 2 h with free access to water before being slaughtered by severing the jugular vein in a slaughterhouse. Carcass weight was determined after removal of the blood, feathers, head, feet, neck, and internal organs, except for the kidneys and lungs. Carcass yield was calculated as the ratio of carcass weight to live BW and expressed as a percentage. The digestive organs, including the crop, proventriculus, gizzard, liver, heart, small intestine, caeca, and colon, were then examined. The weight of each empty organ was recorded and expressed relative to live BW. The lengths of the small intestine, its individual segments, the caeca, and the colon were also measured.

Intestinal histomorphology

On day 35 of the trial, one intestinal sample per replicate was collected for histomorphological analysis, resulting in a total of 24 samples. The digestive tract was divided into the duodenum, jejunum, ileum, caeca, and colon, and the length of each segment was measured. Tissue samples for histomorphological analysis were collected approximately 2 cm from the midpoint of the duodenum, jejunum, and ileum, and fixed in 10% buffered formaldehyde solution. Histological preparations were conducted according to Ariyadi et al. (2019) using haematoxylin and eosin (HE) staining. The sections were examined under a light microscope at 10× magnification using Optilab Viewer software version 2.2 (PT. Miconos Transdata Nusantara, Yogyakarta, Indonesia), and morphometric measurements were performed using Image Raster version 4.0.5 (PT. Miconos Transdata Nusantara, Yogyakarta, Indonesia).

For each intestinal segment, five well-oriented villi were selected and measured, and the average value per bird was used for statistical analysis. Villus height (VH) was measured from the tip of the villus to the villus-crypt junction. Villus width (VW) was measured at the widest point of the villus. Crypt depth (CD) was measured from the villus-crypt junction to the base of the crypt. Villus surface area (VSA) was calculated according to El Sabry and Abd El-Ghany (2021).

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) based on a completely randomised design using IBM SPSS Statistics 26 software (SPSS Inc., Chicago, IL, USA). The pen was considered the experimental unit for carcass characteristics, digestive organ measurements, and intestinal histomorphology. When significant treatment effects were detected ($P < 0.05$), means were separated using Duncan's Multiple Range Test (DMRT).

Additionally, planned pairwise contrasts were performed to evaluate predefined treatment comparisons. Mortality data were further analysed using a generalized linear model (GLM) with a binomial distribution.

Results

Growth performance and carcass trait

Dietary treatments (AB, EO, and ES) did not affect growth performance on day 21 (Table 2). However, mortality was higher in the AB group than in the CO and EO groups during Week 1 ($P < 0.05$; Table 3). In week 2, the AB treatment resulted in the highest mortality among all groups ($P < 0.001$), and this difference remained significant compared with the CO and ES groups at week 5 ($P < 0.001$). At day 35, no significant effects of dietary treatments were observed on BWG, FI, FCR, or PI. Pairwise contrast analysis confirmed higher mortality in the AB group than in the other treatment groups ($P < 0.05$). Although no significant effects were detected for growth performance parameters at day 35, ES tended to improve BWG compared to the control group ($P = 0.06$). Similarly, ES tended to increase PI compared to the CO ($P = 0.07$) and EO ($P = 0.06$) groups. Carcass traits were not affected by dietary treatments (Table 4). However, birds in the ES group tended to have a greater live BW than the control group ($P = 0.07$).

Relative weight of internal organs

On day 21, birds in the AB and ES groups had higher crop weights than those in the EO group ($P < 0.05$; Table 5). Gizzard weight was significantly affected by dietary treatment on both days 21 and 35 ($P < 0.05$). On day 21, the ES group had the highest gizzard weight, whereas the AB group had lower values than the CO and ES groups ($P < 0.01$). Liver weight was also higher in the ES group than in the CO and AB groups on day 21 ($P < 0.05$). On day 35, gizzard weight remained higher in the ES group than in the AB group ($P < 0.05$). Pairwise contrast analysis confirmed greater gizzard weight in the ES group than that in the EO ($P < 0.05$) and AB ($P < 0.01$) groups. Caecum weight was also affected by treatment on day 35, with the EO group showing lower values than the AB group ($P < 0.05$). Pairwise contrast analysis further showed lower caecum weight in the EO group than in the AB and ES groups ($P < 0.05$).

Digestive tract length

The lengths of the digestive tract on days 21 and 35 are presented in Table 6. On day 21, dietary treatments did not significantly affect small intestinal length, although a tendency was observed ($P = 0.08$). Pairwise contrast analysis showed that broilers in the ES group had a longer small intestine than those in the control group ($P < 0.05$). Jejunum length was also increased in the ES group compared to the CO group ($P < 0.05$), which was confirmed by pairwise contrast analysis for both absolute and relative jejunum length ($P < 0.05$). Colon length was affected by dietary treatments ($P < 0.05$), with all supplemented groups (AB, EO, and ES) showing greater values than the CO group ($P < 0.05$). On day 35, no significant effects of dietary treatments were observed on intestinal tract length ($P > 0.05$). Although the AB group showed numerically greater lengths of the small intestine, ileum, and caecum compared to the control, these differences were not statistically significant.

Intestinal histomorphology

In the duodenum, dietary treatments tended to increase the villus-to-crypt ratio ($P < 0.10$; Table 7). Pairwise contrast analysis indicated that AB and ES groups had higher villus-to-crypt ratios than the control group ($P < 0.05$). In the jejunum, dietary treatments significantly affected CD and villus-to-crypt ratio ($P < 0.05$). The AB, EO, and ES groups showed lower CD and higher villus-to-crypt ratios compared to the CO group. In the ileum, dietary treatments significantly influenced villus morphology ($P < 0.05$). The ES group had the shortest villi and the greatest villus width. Pairwise contrast analysis showed that villi were shorter in the ES group than in the CO and AB groups ($P < 0.05$), while the EO group also had shorter villi compared to AB ($P < 0.05$). Villus width was also affected by treatment ($P < 0.05$), with the AB group showing lower values than the CO and ES groups, whereas the ES group had the greatest villus width. Villus surface area was lower in the EO group than in the CO group ($P < 0.05$).

Discussion

Supplementation with EO and EO-PSB did not affect the growth performance of broilers in the present work, contrary to the findings of several previous studies. Khattak et al. (2014) reported that dietary EO blends containing basil, caraway, laurel, lemon, oregano, sage, tea, and thyme increased BW, BWG, and feed efficiency in broilers. Similarly, supplementation with a blend of cinnamaldehyde (200 mg/kg), carvacrol (200 mg/kg), and thymol (100 mg/kg) improved BW, FI, and BWG without affecting feed efficiency (Malhi et al., 2025). Sampath et al. (2025) also reported that a microencapsulated phytogenic feed additive containing EO from oregano, cinnamon, and clove improved broiler performance during the finisher phase and over the entire rearing period, resulting in greater BWG, improved feed efficiency, higher final BW, and lower mortality.

The lack of response observed in the present study may be attributed to differences in EO composition, inclusion levels, and experimental conditions compared to previous studies (Puvača et al., 2013b, 2022). In addition, the absence of significant environmental or pathogenic challenges may have limited the effects of EO supplementation, which are often more evident under suboptimal or stressful conditions. Previous studies have shown that oregano and garlic EO inhibit the growth of *Clostridium* spp., whereas ginger oil and carvacrol supplementation have been associated with improved resistance to necrotic enteritis and growth performance (Kirkpinar et al., 2011; Jerzsele et al., 2012).

Furthermore, in a *Salmonella* challenge model, combined supplementation with EO and PSB reduced pathogen colonisation without affecting growth performance (Cerisuelo et al., 2014). Under *Clostridium perfringens* challenge conditions, the same combination improved growth performance, intestinal morphology, and reduced lesion severity compared to infected controls (Jerzsele et al., 2012). Similar findings have been reported in other challenge studies, where EO–PSB supplementation altered the intestinal microbiota and reduced the adverse effects of necrotic enteritis (Bortoluzzi et al., 2018).

In the current study, the antibiotic-treated group showed higher mortality than the other treatments. Although antibiotics are generally expected to improve health and performance, the reason for the increased mortality observed in this study could not be determined. Previous research has shown that antibiotic supplementation can alter the intestinal microbiota and affect beneficial bacterial populations (Reznichenko et al., 2021; Abbas et al., 2024; Ding et al., 2025; Xu et al., 2025). However, no diagnostic, microbiological, or pathological examinations were performed in the present study to determine whether these factors contributed to the observed mortality. The observed response may be related to differences in experimental conditions, bird sensitivity, or management factors, which could influence the outcome of antibiotic supplementation.

In the present study, birds in the ES group had greater gizzard weight than those in the AB group. Similar results have been reported by Lan et al. (2020a) and Ogwuegbu et al. (2021), who observed increased gizzard weight following SB supplementation. A larger gizzard has been associated with improved intestinal motility and increased cholecystokinin release, which stimulates pancreatic enzyme secretion (Lan et al., 2020b). In contrast, the lower gizzard weight observed in the other treatment groups may be related to reduced microbial populations in the upper gastrointestinal tract (Dehghani-Tafti and Jahanian, 2016). The administration of ES also increased liver weight in broilers. Previous studies have likewise reported higher liver weight following dietary supplementation with EO (Lee et al., 2003; Khosravinia et al., 2013; Abo Ghanima et al., 2021; Oladokun et al., 2021) or PSB (Aghazadeh and TahaYazdi, 2012; Hanim et al., 2023). This response may reflect changes in metabolic activity associated with nutrient metabolism and detoxification However, because liver histology and blood biochemical variables were not evaluated, the physiological significance of the increased liver weight observed in the present study cannot be determined.

In this study, ES supplementation increased jejunum length compared to the control group and tended to increase it in relation to the EO group. A longer jejunum may provide a larger absorptive surface, which could improve nutrient digestion and absorption (Ganjali et al., 2015; Li et al., 2018; Aderibigbe et al., 2020). Earlier studies have shown that EO supplementation can improve nutrient digestibility and absorption (Basmacioğlu Malayoğlu et al., 2010; Pirgozliev et al., 2019; Su et al., 2021), which may contribute to normal development of the digestive tract (Noy and Uni, 2010; Proszkowiec-Weglarz et al., 2022; Abd El- Azeem et al., 2024). Additionally, dietary butyrate supplies energy to epithelial cells and stimulates their proliferation and differentiation while supporting intestinal barrier function (Guilloteau et al., 2010). Infusion of butyrate into the colon has also been shown to exert trophic effects on epithelial cells in the ileum and jejunum, increasing cell proliferation and differentiation and reducing apoptosis in the small intestine. In addition, it reduced apoptosis in the small intestine by regulating gene expression and protein synthesis (Sengupta et al., 2006). These effects may explain the greater relative weight and length of the small intestine reported after PSB supplementation.

In the current study, AB, EO, and EO-PSB treatments reduced crypt depth and increased the jejunal villus height-to-crypt depth (V:C) ratio. Similar effects of EO supplementation on intestinal morphology have been reported previously, and they are recognised as indicators of enhanced intestinal function (Qaisrani et al., 2014). Sun et al. (2015) also observed increased ileal villus height and V:C ratio following EO supplementation, whereas Mohiti-Asli and Ghanaatparast-Rashti (2018) reported reduced jejunal crypt depth and an increased V:C ratio without changes in villus height. These effects may be related antimicrobial properties of EOs, which can influence intestinal microbial populations and nutrient absorption (García et al., 2007; Sarıca et al., 2014; Du et al., 2016; Abd El-Hamid et al., 2024).

PSB supplementation has been associated with improvements in intestinal morphology, particularly increased villus height and the villus height-to-crypt depth ratio in broilers (Kihal et al., 2025). These morphological changes may indicate improved absorptive capacity. PSB has also been reported to improve intestinal morphology and alter the intestinal microbiota, including increasing the abundance of beneficial bacterial genera such as *Bacteroides* and *Lactobacillus* (Jiang et al., 2015; (Moquet et al., 2016;Wu et al., 2016; Sadurní et al., 2022b; Wan et al., 2022). However, because PSB was evaluated only in combination with EO in the present study, its independent contribution cannot be distinguished from the overall effect of ES. Therefore, the role of PSB in the observed responses should be interpreted with caution.

Conclusions

In summary, the combination of essential oil (EO) and protected sodium butyrate (PSB) reduced mortality relative to the antibiotic treatment and affected selected digestive organ development, intestinal length, and gut histomorphology in broilers. However, no significant improvements were observed in growth performance or carcass traits. In contrast, antibiotic supplementation was associated with higher mortality and alterations in selected intestinal parameters. These findings suggest that the EO+PSB combination may be considered a potential alternative to antibiotic growth promoters in improving certain gut-related traits and reducing mortality. Further studies are needed to determine the optimal dosage and ratio of EO and PSB and to evaluate their effects on intestinal microbiota, immune responses, and nutrient utilisation, and disease resistance under commercial production conditions.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Table 1. Ingredients and nutrient composition of experimental broiler diets, %

Ingredient	Starter				Grower				Finisher			
	CO	AB	EO	ES	CO	AB	EO	ES	CO	AB	EO	ES
Maize	55.00	55.00	55.00	55.00	58.12	58.11	58.08	58.08	63.24	63.24	63.21	63.21
Soybean Meal	33.46	33.43	33.17	33.43	27.54	27.52	27.30	27.30	19.88	19.88	19.66	19.66
Corn gluten Meal	2.19	2.21	2.39	2.39	2.70	2.72	2.87	2.87	3.71	3.71	3.85	3.85
Meat bone Meal	2.50	2.50	2.50	2.50	3.50	3.50	3.50	3.50	400	4.00	4.00	4.00
Palm kernel Meal	1.00	1.00	1.00	1.00	1.50	1.50	1.50	1.50	2.00	2.00	2.00	2.00
Bran pollard	-	-	-	-	1.00	1.00	1.00	1.00	1.50	1.50	1.50	1.50
Palm oil	2.15	2.15	2.13	2.13	3.00	3.00	3.00	3.00	3.50	3.50	3.50	3.50
L-Lysine	0.30	0.30	0.31	0.31	0.23	0.23	0.24	0.24	0.24	0.24	0.24	0.24
DL-Methionine	0.32	0.32	0.32	0.32	0.22	0.22	0.22	0.22	0.17	0.17	0.17	0.17
L-Threonine	0.18	0.18	0.18	0.18	0.11	0.11	0.11	0.11	0.08	0.08	0.08	0.08
Limestone	1.07	1.07	1.07	1.07	0.82	0.82	0.82	0.82	0.60	0.60	0.60	0.60
NaCl	0.22	0.22	0.22	0.22	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19
Sodium bicarbonate	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.19	0.19
Sodium humate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calcium propionate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Enzyme	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Toxin binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Additive	0.81	0.81	0.81	0.81	0.26	0.26	0.26	0.26	0.11	0.11	0.11	0.11
Essential oil	-	-	0.10	0.025	-	-	0.10	0.025	-	-	0.10	0.025
Protected sodium butyrate	-	-	-	0.075	-	-	-	0.075	-			0.075
Antibiotic	-	0.01	-	-		0.01	-	-	-	0.01	-	-
Total	100	100	100	100	100	100	100	100	100	100	100	100
Nutrient composition												
Metabolizable energy, kcal/kg	2 960	2 960	2 960	2 960	3 073	3 073	3 073	3 073	3 183	3 183	3 183	3 183
Crude protein	22.79	22.79	22.79	22.79	21.37	21.37	21.37	21.37	19.32	19.32	19.32	19.32
Lysine	1.417	1.417	1.417	1.417	1.240	1.240	1.240	1.240	1.070	1.070	1.070	1.070
Methionine	0.680	0.680	0.680	0.680	0.567	0.567	0.567	0.567	0.499	0.499	0.499	0.499
Methionine + cysteine	1.029	1.029	1.029	1.029	0.896	0.896	0.896	0.896	0.802	0.802	0.802	0.802
Threonine	1.054	1.054	1.054	1.054	0.919	0.919	0.919	0.919	0.796	0.796	0.796	0.796
Calcium	0.804	0.804	0.804	0.804	0.796	0.796	0.796	0.796	0.742	0.742	0.742	0.742
Available phosphorus	0.283	0.283	0.283	0.283	0.326	0.326	0.326	0.326	0.340	0.340	0.340	0.340

CO – basal diet, AB – basal diet mixed with antibiotic 0.1 g/kg diet, EO – basal diet mixed with essential oil 1 g/kg diet, ES – basal diet mixed with essential oil 0.25 g/kg diet and protected sodium butyrate 0.75 g/kg diet

Table 2. Effects of dietary essential oil and protected sodium butyrate on growth performance of male broilers, measured on day 21 and 35

Variables	Treatments				SEM	ANOVA (*P*-value)	Pairwise contrast					
	CO	AB	EO	ES			CO vs AB	CO vs EO	CO vs ES	AB vs EO	AB vs ES	EO vs ES
Day 21												
Body weight gain, g/bird/day	45.3	46.4	45.7	45.6	0.22	0.371	0.099	0.589	0.685	0.251	0.202	0.892
Feed intake, g/bird/day	64.9	64.2	64.9	61.8	0.88	0.603	0.836	0.936	0.268	0.774	0.363	0.236
Feed conversion ratio, g/g	1.43	1.38	1.42	1.36	0.02	0.434	0.348	0.865	0.156	0.436	0.612	0.207
Performance index	331	349	333	350	4.5	0.285	0.171	0.903	0.138	0.209	0.902	0.170
Day 35												
Body weight gain, g/bird/day	59.5	60.3	60.8	61.6	0.38	0.284	0.445	0.218	0.064	0.627	0.253	0.502
Feed intake, g/bird/day	89.4	92.3	92.4	88.6	1.2	0.620	0.428	0.411	0.822	0.976	0.312	0.299
Feed conversion ratio, g/g	1.50	1.53	1.51	1.44	0.02	0.419	0.638	0.792	0.284	0.837	0.130	0.186
Performance index	391	395	390	425	6.6	0.185	0.845	0.930	0.071	0.778	0.103	0.060

CO – basal diet, AB – basal diet mixed with antibiotic 0.1 g/kg diet, EO – basal diet mixed with essential oil 1 g/kg diet, ES – basal diet mixed with essential oil 0.25 g/kg diet and protected sodium butyrate 0.75 g/kg diet; SEM – standard error of the mean; $P > 0.05$ (no statistically significant)

Table 3. Effects of dietary essential oil and protected sodium butyrate on mortality (%) of male broilers

Mortality	Treatment				SEM	p-value
	CO	AB	EO	ES		
Week 1	0.17^{b}	1.0^{a}	0.17^{b}	0.33^{ab}	0.22	0.021
Week 2	0.50^{b}	1.8^{a}	0.50^{b}	0.33^{b}	0.28	<0.001
Week 3	1.0	1.8	1.2	0.67	0.40	0.210
Week 4	2.8	3.3	3.00	2.67	0.33	0.521
Week 5	4.0^{b}	6.0^{a}	4.8^{ab}	3.0^{b}	0.51	<0.001

CO – basal diet, AB – basal diet mixed with antibiotic 0.1 g/kg diet, EO – basal diet mixed with essential oil 1 g/kg diet, ES – basal diet mixed with essential oil 0.25 g/kg diet and protected sodium butyrate 0.75 g/kg diet; SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 4. Effects of dietary essential oil and protected sodium butyrate on carcass trait of male broilers, measured on day 21 and 35

Variables	Treatments				SEM	ANOVA (*P*-value)	Pairwise contrast					
	CO	AB	EO	ES			CO vs AB	CO vs EO	CO vs ES	AB vs EO	AB vs ES	EO vs ES
Day 21												
Live weight, g/bird	997	1018	1005	1001	4.5	0.412	0.118	0.574	0.754	0.302	0.203	0.802
Carcass weight, g	738	748	761	756	9.6	0.851	0.588	0.326	0.624	0.490	0.821	0.882
Dressing carcass, %	73.9	73.5	75.9	75.5	1.0	0.825	0.807	0.478	0.672	0.349	0.514	0.897
Day 35												
Live weight, g/bird	2128	2155	2175	2199	13.2	0.286	0.468	0.216	0.066	0.597	0.244	0.514
Carcass weight, g	1531	1520	1556	1587	14.4	0.392	0.830	0.582	0.296	0.261	0.126	0.340
Dressing carcass, %	72.0	70.7	71.6	72.1	0.78	0.924	0.579	0.865	0.954	0.699	0.541	0.820

CO – basal diet, AB – basal diet mixed with antibiotic 0.1 g/kg diet, EO – basal diet mixed with essential oil 1 g/kg diet, ES – basal diet mixed with essential oil 0.25 g/kg diet and protected sodium butyrate 0.75 g/kg diet; SEM – standard error of the mean; $P > 0.05$ (no statistically significant)

Table 5. Effects of dietary essential oil and protected sodium butyrate on relative weights of internal organs in male broilers, measured on day 21 and 35, %

Variables	Treatments				SEM	ANOVA (*P*-value)	Pairwise contrast					
	CO	AB	EO	ES			CO vs AB	CO vs EO	CO vs ES	AB vs EO	AB vs ES	EO vs ES
Day 21												
Crop	0.28^{ab}	0.32^{a}	0.24^{b}	0.33^{a}	0.01	0.021	0.153	0.204	0.092	0.011	0.778	0.006
Proventriculus	0.69	0.52	0.59	0.65	0.04	0.375	0.103	0.319	0.659	0.500	0.221	0.572
Gizzard	2.3^{a}	1.9^{b}	2.1^{ab}	2.4^{a}	0.06	0.009	0.010	0.271	0.406	0.102	0.001	0.061
Liver	2.1^{b}	2.2^{b}	2.6^{ab}	3.0^{a}	0.11	0.026	0.542	0.257	0.001	0.385	0.001	0.274
Heart	0.61	0.60	0.55	0.66	0.02	0.430	0.821	0.372	0.464	0.459	0.393	0.213
Cecum	0.51	0.58	0.46	0.64	0.04	0.434	0.563	0.676	0.392	0.118	0.641	0.154
Day 35												
Crop	0.35	0.29	0.29	0.26	0.02	0.481	0.284	0.296	0.140	0.977	0.668	0.648
Proventriculus	0.60	0.39	0.52	0.48	0.04	0.359	0.074	0.623	0.325	0.308	0.227	0.765
Gizzard	1.2^{ab}	1.0^{b}	1.1^{ab}	1.3^{a}	0.04	0.024	0.134	0.758	0.084	0.226	0.003	0.046
Liver	2.37	2.46	2.41	2.40	0.08	0.984	0.698	0.849	0.882	0.843	0.810	0.966
Heart	0.46	0.46	0.47	0.47	0.02	0.699	0.534	0.781	0.603	0.729	0.259	0.428
Caecum	0.62^{ab}	0.78^{a}	0.42^{b}	0.74^{ab}	0.05	0.041	0.232	0.122	0.351	0.010	0.784	0.018

CO – basal diet, AB – basal diet mixed with antibiotic 0.1 g/kg diet, EO – basal diet mixed with essential oil 1 g/kg diet, ES – basal diet mixed with essential oil 0.25 g/kg diet and protected sodium butyrate 0.75 g/kg diet; SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 6. Effects of essential oil and protected sodium butyrate on the length of intestinal tracts of male broilers, measured on day 21 and 35

Variables	Treatments				SEM	ANOVA (*P*-value)	Pairwise contrast					
	CO	AB	EO	ES			CO vs AB	CO vs EO	CO vs ES	AB vs EO	AB vs ES	EO vs ES
Day 21												
Small intestine, cm	135	143	141	156	3.0	0.079	0.283	0.453	0.013	0.738	0.122	0.065
Duodenum, cm	24.7	25.3	24.3	25.2	0.47	0.893	0.649	0.820	0.732	0.496	0.909	0.570
Duodenum, %	18.2	17.7	17.3	16.3	0.35	0.268	0.593	0.376	0.060	0.721	0.163	0.290
Jejunum, cm	54.0^{b}	60.8^{ab}	58.2^{ab}	65.8^{a}	1.5	0.033	0.082	0.277	0.005	0.482	0.195	0.053
Jejunum, %	40.3	42.5	41.3	42.2	0.45	0.385	0.117	0.459	0.016	0.474	0.287	0.088
Ileum, cm	55.8	57.0	58.0	64.8	1.5	0.177	0.786	0.615	0.047	0.816	0.080	0.123
Ileum, %	41.2	39.8	41.2	41.5	0.48	0.679	0.372	1.00	0.822	0.372	0.267	0.822
Caecum, cm	25.5	27.0	25.5	28.2	0.64	0.424	0.428	1.00	0.165	0.428	0.536	0.165
Colon, cm	6.3^{b}	8.0^{a}	8.7^{a}	8.3^{a}	0.26	0.002	0.008	<0.001	0.015	0.129	0.651	0.424
Day 35												
Small intestine, cm	176	192	183	186	2.6	0.158	0.029	0.345	0.166	0.180	0.369	0.643
Duodenum, cm	28.3	31.8	29.5	30.2	0.65	0.309	0.070	0.531	0.328	0.216	0.373	0.719
Duodenum, %	16.2	16.7	16.0	16.3	0.26	0.844	0.523	0.831	0.831	0.396	0.669	0.669
Jejunum, cm	75.0	78.1	76.7	78.2	0.97	0.661	0.289	0.573	0.289	0.612	1.00	0.612
Jejunum, %	42.7	40.7	42.3	42.1	0.38	0.304	0.085	0.765	0.655	0.146	0.189	0.881
Ileum, cm	72.3	82.3	76.3	77.4	1.6	0.194	0.035	0.377	0.257	0.191	0.288	0.795
Ileum, %	41.2	43.0	41.8	41.7	0.44	0.585	0.189	0.626	0.715	0.397	0.334	0.903
Caecum, cm	36.0	40.6	38.2	36.7	0.69	0.078	0.018	0.244	0.716	0.181	0.038	0.416
Coloncm	9.5	9.5	8.8	9.5	0.39	0.927	1.00	0.587	1.00	0.587	1.00	0.587

CO – basal diet, AB – basal diet mixed with antibiotic 0.1 g/kg diet, EO – basal diet mixed with essential oil 1 g/kg diet, ES – basal diet mixed with essential oil 0.25 g/kg diet and protected sodium butyrate 0.75 g/kg diet; SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 7. Effects of dietary essential oil and protected sodium butyrate on intestinal histomorphology in male broilers, measured on day 35, μm

Variables	Treatments				SEM	ANOVA (*P*-value)	Pairwise contrast					
	CO	AB	EO	ES			CO vs AB	CO vs EO	CO vs ES	AB vs EO	AB vs ES	EO vs ES
Duodenum												
VH	1014	1093	1062	1119	30.4	0.682	0.390	0.599	0.258	0.732	0.777	0.534
VW	117	107	126	127	4.3	0.295	0.394	0.439	0.391	0.114	0.098	0.930
CD	193	161	169	160	7.0	0.304	0.110	0.224	0.101	0.676	0.963	0.643
V:C	5.3	6.9	6.5	7.2	0.29	0.088	0.047	0.133	0.019	0.579	0.674	0.317
VSA, $10^3 \times \mu m^2$	189	184	208	227	9.8	0.425	0.864	0.498	0.193	0.398	0.145	0.515
Jejunum												
VH	681	760	586	682	28.5	0.193	0.309	0.229	0.980	0.035	0.320	0.220
VW	108	101	111	116	4.8	0.783	0.663	0.815	0.581	0.505	0.329	0.750
CD	253^{a}	165^{b}	141^{b}	153^{b}	14.0	0.008	0.010	0.002	0.004	0.442	0.693	0.704
V:C	2.7^{b}	4.6^{a}	4.4^{a}	4.7^{a}	0.27	0.011	0.001	0.030	0.023	0.769	0.887	0.729
VSA, $10^3 \times \mu m^2$	112	125	102	123	7.2	0.692	0.671	0.322	0.089	0.486	0.964	0.043
Ileum												
VH	594^{ab}	637^{a}	472^{bc}	428^{c}	30.4	0.030	0.557	0.105	0.033	0.034	0.010	0.549
VW	105^{ab}	79.2^{c}	87.0^{bc}	116.8^{a}	4.5	0.003	0.012	0.066	0.208	0.400	0.001	0.005
CD	187	165	136	130	10.9	0.212	0.451	0.098	0.065	0.341	0.245	0.824
V:C	3.3	4.0	3.5	3.6	0.18	0.549	0.171	0.698	0.545	0.315	0.427	0.827
VSA, $10^3 \times \mu m^2$	99.0	80.8	64.6	79.0	5.7	0.212	0.255	0.040	0.213	0.309	0.908	0.364

CO – basal diet, AB – basal diet mixed with antibiotic 0.1 g/kg diet, EO – basal diet mixed with essential oil 1 g/kg diet, ES – basal diet mixed with essential oil 0.25 g/kg diet and protected sodium butyrate 0.75 g/kg diet; VH – villus height, VW – villus width, CD – crypt depth, V:C – villus-crypt ratio, VSA – villus surface area; SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$