

EFFECTS OF CLOVE (*Syzygium aromaticum*) ON PRODUCTIVE PERFORMANCE, NUTRIENTS VALUE AND DIGESTIBILITY, BLOOD LIPID PROFILE, ANTIOXIDANT STATUS AND IMMUNE RESPONSE OF GROWING RABBITS

Marwa Abd Elmonem SULIMAN¹  , Fatma Galal AHMED¹ , Khaled Fahmy EL-KHOLY¹ , Rehab Abd elhay MOHAMED¹  and Lamiaa Fahmy ABDEL-MAWLA² 

¹Utilization of By-product Research Department, Animal Production Research Institute, Agriculture Research Center, 12618 Nady El-sead St., Dokki, Giza, Egypt

²Rabbits, Turkey and waterfowl Breeding Research Department, Animal Production Research Institute, Agriculture Research Center, 12618 Nady El-sead St., Dokki, Giza, Egypt

✉ Email: marwaelaskary@gmail.com

↳ Supporting Information

ABSTRACT: The current study evaluated the effect of feeding clove (*Syzygium aromaticum*) as a natural additive on productive performance, digestibility and nutritive value, antioxidant enzymes activities, and immune response of growing rabbits. A total of 48 New Zealand White (NZW) rabbits aged 6 weeks were randomly allocated to 4 groups (12 rabbits/group). Clove buds powder (CLP) was supplemented at 0.5, 1, and 1.5% of basal diet. Four tested diets formulated to contain basal diet without CLP (treatment 1, T1), 0.5% CLP (T2), 1% CLP (T3), and 1.5% CLP (T4). The animals were provided pelleted diets and fresh water *ad libitum* throughout the experimental period. The rabbits fed diets containing CLP improved FCR ($P=0.007$) and consumed ($P<0.0001$) less than those fed control group. The diet containing 1.5% CLP had the best feed conversion ratio (FCR) value ($P<0.05$). No significant differences were observed among experimental groups in all nutrients digestibility except CP digestibility significantly ($P=0.0261$) increased with 0.5 and 1% CLP groups compared to control group. Blood total lipid (TL) was significantly decreased ($P<0.009$) with increasing the dietary level of CLP, (being 379.17 and 361.11 mg/dl for 1% and 1.5% CLP groups vs. 470.84 for the control group). The catalase and total antioxidant capacity (TAOC) concentrations significantly ($P<0.0001$) increased with CLP groups compared to control group. The immunoglobulins titres (IgG and IgM) improved ($P>0.05$) with rabbits fed CLP diets when compared to those fed the control diet. In conclusion, using CLP as an alternative feed additive in rabbit's diets up to 1.5% without any adverse effect on productive performance and vital activities. The CLP inclusion in rabbit diets decreased feed intake (FI), improved FCR and increased profitability, moreover, had a positive effect on antioxidant enzyme activity and immunity (IgG and IgM) titres.

Keywords: Antioxidant status, Clove, Immune, Performance, Rabbits

INTRODUCTION

Phytogenic as herbs or spices is a natural growth promoters or non-antibiotics growth promoters which are used as feed additives in rabbit diets to improve the productive performance, health status and meat quality (Christaki et al., 2012; Ingweye et al., 2020; Nwachukwu et al., 2021). Moreover, phytogenic help to improving immune system performance in critical situation due to increase the intestinal availability of essential nutrients for absorption, therefore, helping animals to grow better within the framework of their genetic potential (Windisch et al., 2008).

The ban of using antibiotics in livestock nutrition as feed additives attributed to its residual effect which found in final products and increased the consumer's awareness about the health hazards occurs (Anadón et al., 2006; Silveira et al., 2021). Moreover, due to the use of antibiotics in livestock nutrition triggered searching for alternative natural and safe healthy for animals and human when used as feed additives, especially a source of antibiotic (Khamisabadi et al., 2016). Like, herbs are often preferred because they are natural and do not put harmful chemicals into the body (Agrawal et al., 2014). Herbs considered as an alternative feed additive of antibiotics and drug that using in poultry diets to avoid the residual cumulative effect in final poultry products, which negatively affects human health (Ragab, 2012). Clove (*Syzygium aromaticum*) has potent as antioxidant and antimicrobial activities standing out among the other spices (Shan et al., 2005). The clove powder supplementation in broilers diet at 0.5% improved body weight gain and feed conversion ratio (Mahrous et al., 2017). Also, supplementation of 0.50% clove buds and aloe vera leaves improved dressing percentage and breast weight of Japanese quails (Tariq et al., 2015). In rabbit diets added clove a combination with onion, garlic, caraway, fennel, gentian, melissa, peppermint, anise, and oak bark decreased post-weaning mortality rate, improved feed utilization, and enhanced animal performance (Krieg et al., 2009). Sulieman et al. (2007) used clove (*Syzygium aromaticum*) as an antimicrobial, antiseptic, and preservative agent. Furthermore, clove essential oil exhibits a

RESEARCH ARTICLE
 PII: S222877012300001-13
 Received: November 10, 2022
 Revised: January 17, 2023
 Accepted: January 17, 2023

wide range of pharmacological and biological activities such as antioxidant (Gülçin et al., 2012), antifungal (Omidbeygi et al., 2007), and antiprotozoal effects (Machado et al., 2011).

Current study investigated effect of clove (CLP) supplementation in growing rabbit diets on productive performance, nutritive value, blood lipid profile, antioxidant enzymes activity, and immune response.

MATERIALS AND METHODS

The experiment was conducted in Borg El-Arab experiment station, Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Giza, Egypt. The laboratory works were carried out at Utilization of By-products Research Department, APRI, Giza, Egypt. Feed mixing and pelleting processing were prepared at Nobarria feed manufactory, Nobarria experiment station, APRI, Alexandria, Egypt.

Ethical approval

This study was carried out after approved ethically from the APRI, Giza, Egypt under code No. 432429-21-6.

Diets and animals management

Clove buds (*Syzygium aromaticum*) grinded by hammer mill then was taken sample from clove buds powder (CLP) to determine the chemical analysis composition (A.O.A.C., 2000) and total antioxidant capacity (TAOC; Prieto et al., 1999) assayed by spectrophotometer (JENWAY 3600). Rabbit basal diet supplemented with 0.0, 0.5, 1.0 and 1.5% clove buds powder (CLP). Basal diet without CLP supplementation (T1) which considered as a control group, basal diet with 0.5% CLP (T2), 1.0% CLP (T3) and 1.50% CLP (T4). The experimental diets (Table 1) were meet the nutrients requirement of growing rabbits (Lebas, 2004) also, it were to be isonitrogenous and isocaloric.

The experimental New Zealand White (NZW) rabbits were randomly allocated to 4 groups, 12 rabbits for each group. Rabbits weighting averaged 652.81±33.43 g. the experiment lasted 8 weeks (6-14 weeks of rabbit age). The growing rabbits housed in metal battery cage (30 × 35 × 40 cm). The pelleted feed and fresh water provide *ad-libitum* access to separated feeders and automatic nipple fresh water throughout the tested period. The experimental rabbits were kept under the hygienic condition, vaccine program, and management.

Table 1 - Formulation and chemical composition of tested diets

| Ingredients | Control diet | Clove buds powder addition levels | | |
|---|--------------|-----------------------------------|---------|---------|
| | | 0.5% | 1.0% | 1.5% |
| Soybean meal (44% CP) | 17.00 | 17.00 | 17.00 | 17.00 |
| Yellow corn | 13.00 | 13.00 | 13.00 | 13.00 |
| Barley | 12.95 | 12.95 | 12.95 | 12.95 |
| Wheat bran | 16.00 | 16.00 | 16.00 | 16.00 |
| Clover hay | 35.00 | 34.50 | 34.00 | 33.50 |
| Clove buds powder (CLP) | 0.00 | 0.50 | 1.00 | 1.50 |
| DL-methionine | 0.20 | 0.20 | 0.20 | 0.20 |
| Dicalcium phosphate | 2.00 | 2.00 | 2.00 | 2.00 |
| Salt (NaCl) | 0.35 | 0.35 | 0.35 | 0.35 |
| Vitamins and minerals mixture ¹ | 0.30 | 0.30 | 0.30 | 0.30 |
| Anti-coccidia and fungi | 0.20 | 0.20 | 0.20 | 0.20 |
| Molasses | 3.00 | 3.00 | 3.00 | 3.00 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Chemical analysis on DM basis ² | | | | |
| Dry matter (DM, %) | 82.92 | 82.93 | 82.95 | 82.96 |
| Organic matter (OM, %) | 85.94 | 85.93 | 85.91 | 85.90 |
| Crude protein (CP, %) | 17.73 | 17.67 | 17.60 | 17.54 |
| Crude fiber (CF, %) | 14.12 | 14.08 | 14.04 | 14.00 |
| Ether extract (EE, %) | 2.13 | 2.19 | 2.25 | 2.31 |
| Nitrogen free extract (NFE, %) | 54.95 | 55.00 | 55.05 | 55.11 |
| Ash (%) | 5.14 | 5.13 | 5.13 | 5.12 |
| Digestible energy (DE, kcal/kg) ³ | 2605.44 | 2607.98 | 2610.54 | 2613.09 |

¹ Commercial vitamin and mineral premix contained (per 3 kg premix) vit. A 12000 000 IU, vit. D3 3000 000 IU, vit. E 10 000 mg, vit. K3 2000 mg, vit. B1 1000 mg, vit. B2 5000 mg, vit. B6 1500mg, vit. B12 10 mg, pantothenic acid 10 000 mg, nicotinic acid 30 000 mg, folic acid 1000 mg, biotin 75 mg, copper 4000 mg, manganese 80 000 mg, zinc 50 000 mg, iron 30 000 mg, iodine 500 mg, selenium 100 mg and cobalt 100 mg. ²NRC (1977). ³Digestible Energy (kcal/kg) = 4.36-0.049 × [28.924 + 0.657 (CF %)] according to Cheeke, (1987).

Productive performance measurements

Live body weight (BW, g/rabbit/day) of rabbits and feed intake (FI, g/rabbit/day) were recorded weekly. Then the feed conversion ratio (FCR, g feed: g gain) were calculated over an experimental period. The BW, FI, and FCR were calculated on per cage basis and then average by treatment.

Digestion trial

According to Perenze et al. (1995), 20 rabbits divided randomly into 4 groups (5 rabbits/group) to execution the digestion trial. Rabbits were allocated in metabolic cages (56 × 38 × 28 cm). The feces were collected daily before the morning meal. The fresh feces were weighed then dried in air-dry oven at 60 °C for 24 hour. The diets and dried feces ground samples used to estimate the moisture, ash, nitrogen, ether extract, and crude fiber (A.O.A.C., 2000). Those data were used to calculate the digestion coefficient of nutrients, nutritive value (Fekete, 1985), and digestible energy (Schneider and Flatt, 1975) for each tested diets.

Blood lipid profile, antioxidant enzymes activity, and immune response

The blood samples were collected during slaughtering time from five rabbits which randomly selected from each treatment at the end of growing period. The samples were collected in heparinized tubes and centrifuged at 3000 rpm for 20 minute, then transferred the plasma to tubes and stored at -20 °C till biochemical analysis. Plasma total lipids was determined according to Frings and Dunn (1970), cholesterol was estimated according to Young (1997), LDL cholesterol was determined according to Assmann et al. (1984) and HDL cholesterol was determined according to Lopez et al. (1977). The antioxidant enzymes as catalase concentration and total antioxidant capacity were determined according to Góth (1991) and Fischer et al. (2006). Immunoglobulin (IgG and IgM) responses were estimated according to Van der Zipp et al. (1983). All measurements were assayed by colorimetric methods. All kits were purchased from Bio-diagnostic Co, Egypt

Economic profit

Economic efficiency was calculated as a ratio between the return of weight gain and the cost of feed intake. The price of ingredients and selling of one kg live weight of rabbits (\$2.55/kg) was calculated according to the price in local market at the time of experiment.

Statistical analysis

All data were subject to one-way analysis of variance (ANOVA). The data obtained herein were analyzed by the GLM procedure of SAS (2004, USA). Duncan's multiple range test (1955) was performed to separate means and significance accepted at P≤0.05.

RESULTS AND DISCUSSION

Nutritional analysis and total antioxidant of CLP

The nutritional analysis and total antioxidant capacity of CLP are presented in Table 2. The DM, OM, CP, CF, EE, NFE, ash, and DE values of CLP were 91.40, 94.36, 9.17, 13.50, 6.40, 64.80, 6.17%, and 2508.12kcal/kg, respectively. In contrary, the chemical analysis of clove buds were 90, 1.20, 20.10, 12.10 and 5.4% for DM, CP, CF, EE and ash, respectively (Suliman et al., 2007); 85.20, 12.40, 17.50, 16.20 and 12.60%, respectively (Sulaiman and Anas, 2017).

The total antioxidant capacity (TAC) content of CLP was 1069.20 mg/100g (ascorbic acid equivalent). This TAC value is higher than those obtained by Ahmed et al. (2022) who found the TAC as form 2,2-diphenyl-1-picrylhydrazyl (DPPH%) radical scavenging activity in CLP was 83.90 mg/100g. However, Anita et al. (2015) who found the oxidized, reduced, and total ascorbate were 8084.40, 8014, and 6098.50 mg/100g dry wt., respectively. The DPPH scavenging of antioxidant content of CLP was 13660 mg/100mg according to Turgay and Esen (2015). The polyphenols and antioxidant content of CLP is higher than those in other spices (Pérez-Jiménez et al., 2010). The TAC in clove or their extract can promote health (Abo El-maati et al., 2016). Furthermore, it prevents the oxidation of lipids by chelating metal ions or inhibits the propagation reaction being hydrogen / electron donor (Shobana and Naidu, 2000). The active ingredients of clove buds and lemon balm extracts are able to scavenge the free radicals in *in vitro* trail (Petrovic et al., 2012).

Table 2 - Chemical analysis and total antioxidant capacity of clove powder

| Items | Clove buds powder (CLP) |
|--|-------------------------|
| Chemical analysis on DM basis | |
| Dry matter (DM, %) | 91.40 |
| Organic matter (OM, %) | 94.36 |
| Crude protein (CP, %) | 9.17 |
| Crude fiber (CF, %) | 13.50 |
| Ether extract (EE, %) | 6.40 |
| Nitrogen free extract (NFE, %) | 64.80 |
| Ash (%) | 6.17 |
| Digestible energy (DE, kcal/kg) ¹ | 2508.12 |
| Total antioxidant capacity mg/100 g (ascorbic acid equivalent) | |
| TAOC | 1069.20 |

TAC: Total antioxidant capacity; ¹Digestible Energy (kcal/kg) = 4.36-0.049 × [28.924 + 0.657 (CF %)] according to Cheeke, (1987).

Growth performance

The effect of CLP supplementation on growing rabbit's performance is presented in Table 3. No significant differences in FBW ($P=0.7092$) and BWG ($P=0.6400$) were found between the control group (T1) and treatment groups (T2-T4). Final body weight (FBW, g) and body weight gain (BWG, g/day/rabbit) increased with groups fed CLP more than 0.5% level addition ($P>0.05$). However, FI (g/rabbit/day) and FCR (g, feed: g, gain) were significantly ($P<0.0001$ and $P=0.007$, respectively) affected by inclusion of dietary CLP. Rabbits in dietary CLP treatments (T2, T3, and T4) decreased ($P<0.0001$) the FI consuming and improved ($P<0.0078$) FCR during the growing period as compared with control treatment (T1). The dietary 1.5% CLP group had the lowest FCR value (T4, 3.89), which was comparable to the control (T1, 4.70) and better than the 0.5% (T2, 4.15) and 1.0% (T3, 4.28) dietary CLP. The FCR of broiler at finisher period was improved ($P<0.05$) with clove essential oil (455ppm) addition (Mehar et al., 2014). Similarly with Petrovic et al. (2012) who showed slightly improvement ($P>0.05$) in broilers performance (BW, FI and FCR) which fed diets supplemented 1% clove buds with 0.2% lemon balm extract. Mahrous et al. (2017) observed no significant differences in broilers performance indices (FBW, BWG, FI, and FCR) fed diets supplemented 0.5, 1.0, and 1.5% clove buds.

In present study, the improvements in FBW ($P=0.7092$), BWG ($P=0.6400$) and FCR ($P<0.05$) with increasing level of CLP than 0.5% due to increase the diets content of growth promoters properties such as antimicrobial (Dorman et al., 2000). Many studies confirmed the positive effect of spices or their active components on the digestion process, wherein they activate bile salts secretion and digestive enzyme activities in the intestinal mucosa and pancreas (Hernández et al., 2004) which reflect to broilers productive performance (Jang et al., 2007). In contrary, Al-Mufarrej (2019) who reported that negative effect of final live body ($P<0.05$) for broiler fed clove supplemented more than 2%. Hussein et al. (2019) added 1.5 ml clove oil/kg of Japanese quails diet, increased ($P<0.05$) performance in terms of BWG and FI, with no improvement ($P>0.05$) in FCR.

Table 3 - Effect of clove buds powder addition on growing rabbit's performance

| Items | IBW (g) | FBW (g) | BWG (g/rabbit/day) | FI (g/rabbit/day) | FCR (g.feed : g.gain) |
|---------------|--------------|----------------|-----------------------|---------------------------|--------------------------|
| T1 (0.0% CLP) | 650.83±35.17 | 2251.67±53.66 | 28.60±1.11 | 134.28 ±1.86 ^a | 4.70±0.18 ^a |
| T2 (0.5% CLP) | 655.42±32.40 | 2240.42±58.37 | 28.30±0.77 | 117.44±2.37 ^c | 4.15±0.11 ^b |
| T3 (1.0% CLP) | 651.67±32.90 | 2307.50±47.02 | 29.57±0.67 | 126.48±1.62 ^b | 4.28±0.10 ^b |
| T4 (1.5% CLP) | 653.33±33.25 | 2344.17±109.23 | 30.19±1.78 | 117.64±2.53 ^c | 3.89±0.20 ^b |
| P-value | 0.9997 | 0.7092 | 0.6400 | <0.0001 | 0.0078 |

Mean values with different superscript letters in the same column are significantly different ($P<0.05$); CLP: clove buds powder, IBW: initial body weight, FBW: final body weight, BWG: body weight gain, FI: feed intake, FCR: feed conversion ratio

In vitro digestibility and nutritive values

The effect of CLP feeding on digestibility and nutritive values are presented in Table 4. There were slight increase ($P>0.05$) in digestion coefficients percentage of dry matter (DM; $P=0.3476$), organic matter (OM; $P=0.2883$), crude fiber (CF; $P=0.1507$), ether extract (EE; $P=0.4753$), nitrogen free extract (NFE; $P=0.1507$), also, nutritive value as a total digestible nutrients (TDN%, $P=0.1107$) and digestible energy (DE, Kcal/kg, $P=0.1106$) with 0.5 and 1.0% CLP groups compared to control group. Percentage of crude protein (CP) digestibility significantly ($P=0.0261$) increased with 0.5 and 1.0% CLP groups compared to control group. The diet containing 0.5% CLP recorded the best digestibility for all nutrients except % of NFE digestibility. Percentage of digestible crude protein (DCP) had significantly ($P<0.0001$) affected by dietary CLP addition. Percentage of DCP significantly ($P<0.05$) improved by 9.39% with 1.5%CLP addition, but 1.0% CLP inclusion significantly ($P<0.05$) decreased DCP% by 8.23% with relative control group. No significant difference in DCP% was found between 0.50% CLP group and the control group.

The results agreed with Dalkiliç and Güler (2009) who found DM, CP, and EE digestibilities significantly ($P<0.05$) improved by clove extract level up to 400 ppm in in broiler diets. Generally, when looking at either rabbit's performance (Table 3), the FCR improved and FI decreased or nutrient digestibility (Table 4), CP% and DCP% enhanced by dietary CLP addition. This refers to herbs bioactive substances that help to regulate the FI in animals by improving the flavor and regulate the functioning of digestive system (Mirzaei -Aghsaghali, 2012). Moreover, inhibit or enhance metabolism, shape of the sensory, and dietary properties of animal products (Meineri et al., 2010). The spices such as pepper, cinnamon, and clove stimulate the secretion of pancreatic enzymes (lipases, amylases, and proteases), and increase the activity of digestive enzymes of gastric (Srinivasan, 2005).

Table 4 - Digestibility and nutritive values of growing rabbits

| Items | Digestion coefficients (%) | | | | | | Nutritive value | | |
|---------------|----------------------------|------------|--------------------------|------------|------------|------------|-------------------------|------------|-------------------------|
| | DM | OM | CP | CF | EE | NFE | DCP % | TDN % | DE Kcal/kg ¹ |
| T1 (0.0% CLP) | 68.05±1.25 | 71.17±1.32 | 67.66±0.71 ^b | 40.57±1.69 | 89.32±1.35 | 72.17±1.24 | 12.46±0.13 ^b | 68.43±0.86 | 3031.30±38.47 |
| T2 (0.5% CLP) | 69.90±3.91 | 72.74±3.59 | 75.71±2.47 ^a | 58.60±5.27 | 91.45±1.01 | 71.31±3.67 | 12.29±0.14 ^b | 68.63±2.61 | 3040.26±115.78 |
| T3 (1.0% CLP) | 68.14±3.97 | 72.62±3.13 | 73.26±0.91 ^a | 42.54±7.93 | 90.34±3.31 | 74.12±2.68 | 11.33±0.08 ^c | 69.08±2.52 | 3060.18±111.79 |
| T4 (1.5% CLP) | 61.43±3.34 | 64.77±3.67 | 71.62±1.08 ^{ab} | 47.85±4.32 | 85.88±3.39 | 59.98±4.76 | 13.63±0.21 ^a | 60.66±3.09 | 2687.35±136.70 |
| P-value | 0.3476 | 0.2883 | 0.0261 | 0.1507 | 0.4753 | 0.1507 | <0.0001 | 0.1107 | 0.1106 |

Mean values with different superscript letters in the same column are significantly different (P<0.05); CLP: clove buds powder, DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fiber, EE: ether extract, NFE: nitrogen free extract, DCP: digestible crude protein, TDN: total digestible nitrogen, DE: digestible energy. ¹DE (kcal/kg) =TDN x 44.3 (Schneider and Flatt, 1975).

Table 5 – Blood lipid profile, antioxidant enzymes activity, and immunoglobulin titres of growing rabbits

| Items | Blood lipid profile | | | | Antioxidant enzymes activity | | Immunoglobulin titres | |
|---------------|----------------------------|---------------------|-------------|-------------|------------------------------|------------------------|-----------------------|-------------|
| | TL (mg/dl) | Cholesterol (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | Catalase (U/L) | TAOC (mM/L) | IgG | IgM |
| T1 (0.0% CLP) | 470.84±8.82 ^a | 138.35±5.40 | 25.95±0.55 | 98.775±4.23 | 134.55±3.55 ^c | 1.62±0.01 ^d | 296.00±30.60 | 55.50±8.37 |
| T2 (0.5% CLP) | 425.00±25.66 ^{ab} | 174.75±7.01 | 28.65±1.65 | 74.40±4.00 | 146.90±3.58 ^c | 1.68±0.01 ^c | 453.50±34.92 | 58.50±21.65 |
| T3 (1.0% CLP) | 379.17±8.82 ^{bc} | 151.40±16.17 | 30.05±1.47 | 73.52±8.41 | 162.95±2.86 ^b | 1.78±0.01 ^b | 476.50±28.58 | 65.50±10.68 |
| T4 (1.5% CLP) | 361.11±20.85 ^c | 157.95±7.01 | 31.00±3.00 | 69.84±17.14 | 218.50±6.41 ^a | 1.83±0.01 ^a | 719.00±192.26 | 76.50±10.10 |
| P-value | <0.0090 | 0.1400 | 0.3220 | 0.2320 | <0.0001 | <0.0001 | 0.0910 | 0.7170 |

Mean values with different superscript letters in the same column are significantly different (P<0.05). CLP: clove buds powder, TL: total lipids, HDL: high density lipoprotein, LDL: low density lipoprotein, TAOC: total antioxidant capacity, IgG: immunoglobulin G, IgM: immunoglobulin M.

Blood lipid profile, antioxidant enzymes activity and immune response in plasma

Blood lipid profile is shown in Table 5. Total lipid (TL) concentrations significantly ($P=0.009$) decreased with increasing the levels of CLP supplementation. However, no significant differences were observed between tested groups in cholesterol ($P=0.1400$), HDL ($P=0.3220$), and LDL ($P=0.2320$) values. Both cholesterol and HDL concentrations increased ($P>0.05$) with CLP diets, while LDL values were insignificantly lower ($P>0.05$) with rabbits fed CLP diets than control group. Addition of CLP by 0.5, 1.0 and 1.5% increased ($P>0.05$) HDL concentrations (10.40, 15.80, and 19.46%, respectively), however, decreased LDL concentrations (24.68, 25.60 and 29.30%, respectively). As the same trend, by increasing clove essential oil levels in Japanese quail diets, the concentrations of HDL increased ($P<0.05$) and LDL decreased ($P<0.05$) in plasma (Hussein et al., 2019). The decreasing of LDL in blood may due to bioactive substrate (Eugenol) in CLP which plays a vital role in reducing LDL concentrations (Harb et al., 2019). In broilers plasma, no significant differences ($P>0.05$) in concentrations of TL, total cholesterol, LDL and HDL due to feeding on 1% clove buds in diet and 0.2% lemon balm extract in drinking water (Petrovic et al., 2012). In contrast, total cholesterol significantly ($P<0.05$) decreased with supplementation 0.5, 1.0 and 1.5 g clove bud /kg broiler diets (Mahrous et al., 2017).

Antioxidant enzymes activity are presented in Table 5. Dietary CLP supplementation significantly increased ($P<0.05$) catalase and TAOC concentrations in growing rabbits plasma when compared with those in the control group. Rabbits fed on diet including 1.5% CLP gave the highest ($P<0.05$) catalase and TAOC concentrations (218.50 and 1.83, respectively) with compared to rabbits in control diet (134.55 and 1.62, respectively). Similarly, catalase concentration in quails blood significantly ($P<0.05$) increased with diets containing clove essential oils (Hussein et al., 2019). The improvement of catalase and TAOC concentrations in blood are attributed to eugenol (bioactive substrate) that forms iron-oxygen chelate complex through its allyl group and maintains iron and copper in their reduced forms (Ito et al., 2005). Moreover, clove supplementation can prevent hydroxyl radical's synthesis (the secondary products of lipid peroxidation; like clove oil may serve as an effective antioxidant even at the later stages of lipid peroxidation during beta-oxidation process (Jirovetz et al., 2006). In this study, antioxidant status improved ($P<0.0001$) by feeding growing rabbits on highest CLP level (1.5%), this improvement is due to clove active substances with the antioxidant properties (Dragland et al., 2003). A slightly increased in antioxidants (superoxide dismutase and glutathione peroxidase) due to feeding broilers on 1% clove buds and 0.2% lemon balm extract in drinking water (Petrovic et al., 2012).

Immunoglobulin responses are illustrated in Table 5. The immunoglobulin titres (IgG and IgM) recorded insignificantly ($P=0.0910$ and $P=0.7170$, respectively) improvement with CLP rabbit groups when compared to those in control group. Rabbits fed 1.5%CLP recorded the highest IgG and IgM values compared to the other tested groups including the control group. Furthermore, the concentrations of plasma IgG and IgM significantly ($P<0.05$) increased in broiler chickens (5 weeks of age) fed diets supplemented with 1.0 and 1.5% CLP (Mahrous et al., 2017). The improvement of IgG and IgM may be due to that clove act as additional bonds with immunoglobulin molecules at the Fc receptors, which stimulated the immune response (Ahmed et al., 2013).

Economic profit

Profitability and economic efficiency of tested diets are showed in Table 6. According the productive performance of rabbits (Table 3) showed improving in FBW (g) for rabbit's groups fed 1.0 and 1.5% CLP supplementation also, FCR improved with all CLP supplementation groups. The incoming selling price per rabbit recorded increasing with 1.5% CLP (\$5.98) followed by 1.0% CLP (\$5.88) supplementation. The net revenue and economic efficiency showed improving with all CLP supplementation levels. The best economic efficiency of tested diets showed with 1.5%CLP (270.63). Monsi and Onicchi (1991) found that addition of chamomile powder to chicken diets reduced the cost of diets. Karangiya et al. (2016) found that the spices (ginger) supplementation in broiler diets significantly increased incoming from birds selling and feed cost during whole test period while, decreasing the return over feed cost.

Table 6 - The economic profit for growing rabbit tested diets

| Items | Clove buds powder levels | | | |
|----------------------------------|--------------------------|--------|--------|--------|
| | 0.0% | 0.5% | 1.0% | 1.5% |
| Total average weight (kg) | 2.25 | 2.24 | 2.31 | 2.34 |
| Price of one kg body weight (\$) | 2.55 | 2.55 | 2.55 | 2.55 |
| Selling price/rabbit (\$) | 5.74 | 5.71 | 5.88 | 5.98 |
| Total feed intake (kg) | 7.52 | 6.58 | 7.08 | 6.60 |
| Price/kg feed (\$) | 0.36 | 0.32 | 0.28 | 0.24 |
| Total feed cost/rabbit (\$) | 2.70 | 2.10 | 1.98 | 1.60 |
| Net revenue (\$)¹ | 3.04 | 3.61 | 3.90 | 4.38 |
| Economic efficiency² | 112.91 | 174.23 | 196.10 | 270.63 |

¹Net revenue = selling price/rabbit (\$) – total feed cost/rabbit (\$); ²Economical efficiency (%) = (Net revenue / total feed cost/rabbit (\$)) x 100

CONCLUSION

It can conclude that using clove buds powder (CLP) as natural supplement in rabbit diets up to 1.5% without any adverse effect on productive performance and vital activities during growing period from 6-14 weeks of age. The supplementation of CLP in growing rabbit diets improved FCR and decreased FI, moreover, had been positive effect on antioxidant enzymes activity and immunity (IgG and IgM titres). Finally, using the CLP supplementation improved the net revenue and economic efficiency, especially at level of 1.5% CLP.

DECLARATIONS

Corresponding author

E-mail: marwaelaskary@gmail.com

Authors' contribution

M. A. Suliman designed the experiment and drafted the manuscript; F.G. Ahmed collaborated the statistical analysis and participated in manuscript review; Kh. F. El-Kholy participated in manuscript review; R. A. Mohamed performed the practical part and laboratory analysis; L. Abdel-Mawla collaborated the laboratory analysis. All authors read and approved the final manuscript.

Conflicts of interests

The authors have declared that no competing interest exists.

REFERENCES

- Abo El-maati MF, Mahgoub SA, Labib SM, Al-Gaby AM and Ramadan NF (2016). Phenolic extracts of clove (*Syzygium aromaticum*) with novel antioxidant and antibacterial activities. *European Journal of Integrative Medicine*, 8(4):494–504. DOI: <https://doi.org/10.1016/j.eujim.2016.02.006>
- Agrawal M, Agrawal S, Rastogi R., Singh P, Adyanthaya BR, Gupta HL (2014). A review on uses of clove in oral and general health. *Indian Journal Research Pharmacy Biotechnology*, 2(4): 1321-1324. [Article link](#)
- Ahmed IAM, Babiker EE, Al-Juhaimi FY and Bekhit A A (2022). Clove polyphenolic compounds improve the microbiological status, lipid stability, and sensory attributes of beef burgers during cold storage. *Antioxidants*, 11:1354-1371. DOI: <https://doi.org/10.3390/antiox11071354>
- Ahmed ST, Hossain ME, Kim GM, Hwang JA, Ji H and Yang CJ (2013). Effects of resveratrol and essential oils on growth performance immunity, digestibility and fecal microbial shedding in challenged piglets. *Asian-Australas Journal of Animal Science*, 26(5): 683-690. DOI: <https://doi.org/10.5713/ajas.2012.12683>
- Al-Mufarrej SI, Fazea EH, Al-Baadani HH and Qaid MM (2019). Effects of clove powder supplementation on performance, blood biochemistry, and immune responses in broiler chickens. *South African Journal of Animal Science*, 49 (5):835-844. DOI: <https://doi.org/10.4314/sajas.v49i5.6>
- Anadón A (2006). WS14 The EU ban of antibiotics as feed additives: alternatives and consumer safety. *Journal of Veterinary Pharmacology and Therapeutics*, 29:41-44. DOI: <https://doi.org/10.1111/j.1365-2885.2006.00775.2.x>
- Anita D, Avtar S and Ritu M (2015). Antioxidants of clove (*syzygium aromaticum*) prevent metal induced oxidative damage of biomolecules. *International Research Journal of Pharmacy*, 6(4):273-278. DOI: <https://doi.org/10.7897/2230-8407.06460>
- Assmann G, Jabs HU, Kohnert U, Nolte W and Schriewer H (1984). LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinylsulfate. *Clinica Chimica Acta*, 140(1):77-83. DOI: [https://doi.org/10.1016/0009-8981\(84\)90153-0](https://doi.org/10.1016/0009-8981(84)90153-0)
- AOAC (2000). Association of Official Analytical Chemists. Official Methods of Analysis. 17th Edition, Washington, D.C., USA. pp.771.
- Christaki E, Bonos E, Giannenas I and Florou-Paneri P (2012). Aromatic plants as a source of bioactive compounds. *Agriculture*, 2(3): 228-243. DOI: <https://doi.org/10.3390/agriculture2030228>
- Dalkılıç B and Güler T (2009). The Effects of Clove Extract Supplementation on Performance and Digestibility of Nutrients in Broilers. *Firat Üniversitesi Sağlık Bilimleri Dergisi*, 23(3):161-166. Link: http://veteriner.fusabil.org/pdf/pdf_FUSABIL_677.pdf
- Dorman HJD, Surai P and Deans SG (2000). In vitro antioxidant activity of a number of plant essential oils and phytoconstituents. *Journal of Essential Oil Research*, 12; 241–248. DOI: <https://doi.org/10.1080/10412905.2000.9699508>
- Dragland S, Senoo H, Wake K, Holte K and Blomhoff R (2003). Several Culinary and Medicinal Herbs are Important Sources of Dietary Antioxidants. *Journal of Nutrition*, 133: 1286–1290. DOI: <https://doi.org/10.1093/jn/133.5.1286>
- Duncan DB (1955). Multiple Range and Multiple F-Test. *Biometrics*, 11:1-42. DOI: <https://doi.org/10.2307/3001478>
- Fekete S (1985). Rabbit feeds and feeding with special regard to tropical condition. *Journal of Applied Rabbit Research*, 8:167-173. <https://www.worldcat.org/title/journal-of-applied-rabbit-research/oclc/476125850>
- Fischer M AJG, Gransier TJ M, Beckers LMG, Bekers O, Bast A and Haenen GR MM (2006). Determination of antioxidant capacity in blood. *Clinical Chemistry and Laboratory Medicine*, 43(7):735-740. DOI: <https://doi.org/10.1515/CCLM.2005.125>
- Frings, CS and Dunn, RT (1970). A colorimetric method for determination of total serum lipids based on the sulfo-phospho-vanillin reaction. *American Journal of Clinical Pathology*, 3(1): 89-91, DOI: <https://doi.org/10.1093/ajcp/53.1.89>
- Góth L (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*, 196(2-3): 143-151. Link: [https://doi.org/10.1016/0009-8981\(91\)90067-M](https://doi.org/10.1016/0009-8981(91)90067-M)
- Gülçin I, Elmastaş M and Aboul-Enein HY (2012). Antioxidant activity of clove oil—A powerful antioxidant source. *Arabian Journal Chemistry*, 5(4): 489–499. DOI: <https://doi.org/10.1016/j.arabjc.2010.09.016>

- Harb Amani A; Bustanji YK, Almasri IM and Abdalla SS (2019). Eugenol Reduces LDL Cholesterol and Hepatic Steatosis in Hypercholesterolemic Rats by Modulating TRPV1 Receptor. *Scientific Reports*, 9: 14003, DOI: <https://doi.org/10.1038/s41598-019-50352-4>
- Hernández F, Madrid J, García V, Orengo J and Megias MD (2004). Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poultry Science*, 83(2):169–174. DOI: <https://doi.org/10.1093/ps/83.2.169>
- Hussein MMA, Abd El-Hack ME, Mahgoub SA, Saadeldin IM and Swelum AA (2019). Effects of clove (*Syzygium aromaticum*) oil on quail growth, carcass traits, blood components, meat quality, and intestinal microbiota. *Poultry Science*, 98:319-329. DOI: <https://doi.org/10.3382/ps/pey348>
- Ingweye JN, Anaele O, Ologbose FI (2020). Response of rabbit bucks to diets containing Aidan (*Tetrapleura tetraptera*) as feed additive. *Animal Research International*, 17(2):3691-3705. <https://www.ajol.info/index.php/ari/article/view/199329>
- Ito M, Murakami K and Yoshino M (2005). Antioxidant action of eugenol compounds: role of metal ion in the inhibition of lipid peroxidation. *Food and Chemical Toxicology*, 43(3):461–466. DOI: <https://doi.org/10.1016/j.fct.2004.11.019>
- Jang I S, Ko YH, Kang SY and Lee CY (2007). Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. *Animal Feed Science and Technology*, 134(3-4):304–315. DOI: <https://doi.org/10.1016/j.anifeedsci.2006.06.009>
- Jirovetz L, Buchbauer G, Stoilova I, Stoyanova A, Krastanov A and Schmidt E (2006). Chemical composition and antioxidant properties of clove leaf essential oil. *Journal of Agricultural and Food Chemistry*, 54:6303–6307. DOI: <https://doi.org/10.1021/jf060608c>
- Karangiya VK, Savsani HH, Patil SS, Garg DD, Murthy KS, Ribadiya NK and Vekariya SJ (2016). Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. *Veterinary World*, 9(3):245-250. DOI: <https://doi.org/10.14202/vetworld.2016.245-250>
- Khamisabadi H, Kafizadeh F and Charaien B (2016). Effect of thyme (*Thymus vulgaris*) or peppermint (*Mentha piperita*) on performance, digestibility and blood metabolites of fattening Sanjabi lambs. *Biharean biologist*, 10 (2): 118-122. Link: http://biozoojournals.ro/bihbiol/cont/v10n2/bb_e151408_Khamisabadi.pdf
- Krieg R, Vahjen W, Awad W, Sysel M, Kroeger S, Zocher E, Hulan HW, Aarndt G and Zentek J (2009). Performance, digestive disorders and the intestinal microbiota in weaning rabbits are affected by a herbal feed additive. *World Rabbit Science*, 17:87 – 95. DOI: <https://doi.org/10.4995/wrs.2009.662>
- Lebas F (2004). Reflections on rabbit nutrition with special emphasis on feed ingredients utilization. In: Becerril, C.M. and Pro, A (Editors) *Proceedings of the 8th World Rabbit Congress*, Puebla. Colegio de Postgraduados, Montecillo, Spain, pp. 686-736. https://www.researchgate.net/publication/287407223_Reflections_on_rabbit_nutrition_with_a_special_emphasis_on_feed_ingredients_utilization
- Lopez V.MF, Stone P, Ellis S , Colwell JA (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical Chemistry*, 23(5):882-884. DOI: <https://doi.org/10.1093/clinchem/23.5.882>
- Machado M, Dinis AM, Salgueiro L, Custódio JB, Cavaleiro, C and Sousa MC (2011). Anti-giardia activity of *Syzygium aromaticum* essential oil and eugenol: Effects on growth, viability, adherence and ultrastructure. *Experimental Parasitology*, 127: 732–739. DOI: <https://doi.org/10.1016/j.exppara.2011.01.011>
- Mahrous Heba S, El-Far AH, Sadek KM, Abdel-Latif Mervat A (2017). Effects of Different Levels of Clove Bud (*Syzygium Aromaticum*) Dietary supplementation on immunity, antioxidant status, and performance in broiler chickens. *Alexandria Journal of Veterinary Sciences*, 54(2): 29-39. DOI: <https://doi.org/10.5455/ajvs.272231>
- Mehr MA, Hassanabadi A, Moghaddam HN and Kermanshahi H (2014). Supplementation of clove essential oils and probiotic on blood components, lymphoid organs and immune response in broiler chickens. *Research Opinions in Animal and Veterinary Science*, 4:117–122. [Article Link](#)
- Meineri G, Cornale P, Tassone S and Peiretti PG (2010). Effects of Chia (*Salvia hispanica* L.) seed supplementation on rabbit meat quality, oxidative stability and sensory traits. *Italian Journal of Animal Science*, 9(1): 45–49. DOI: <https://doi.org/10.4081/ijas.2010.e10>
- Mirzaei-Aghsaghali A (2012). Importance of medical herbs in animal feeding: A review. *Annals of Biological Research*, 3(2), 918–923. Link: <https://www.scholarsresearchlibrary.com/articles/importance-of-medical-herbs-in-animal-feeding-a-review.pdf>
- Monsi A and Onicichi DO (1991). Effects of ascorbic acid supplementation on ejaculate semen characteristics of broiler breeder chickens under hot and humid tropical conditions. *Animal Feed Science and Technology*, 34(1-2): 114-146. DOI: [https://doi.org/10.1016/0377-8401\(94\)90197-X](https://doi.org/10.1016/0377-8401(94)90197-X)
- NRC (1977). National Research Council. *Nutrient requirements of rabbits*. National Academy of Science, Washington, DC., USA, pp 35.
- Nwachukwu CU, Aliyu KI, Ewuola EO (2021). Growth indices, intestinal histomorphology, and blood profile of rabbits fed probiotics-and prebiotics-supplemented diets. *Translational Animal Science*, 5(3): txab 096. <https://doi.org/10.1093/tas/txab096>
- Omidbeygi M, Barzegar M, Hamidi Z and Naghdibadi H. (2007). Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus Xavus* in liquid medium and tomato paste. *Food Control*, 18(12):1518–1523 DOI: <https://doi.org/10.1016/j.foodcont.2006.12.003>
- Perenz JM, Lebas F, Gidenne T, Mertens L, Xiccato G, Parigi-Bini R, Dalle ZA, Cossu ME, Carazzolo A, Villamide MJ, Carabaño R, Fraga MJ, Ramos MA, Cervera C, Blas E, Fernandez J, Falcanoe CL, Bengala A and Freire J (1995). European reference method for in vivo determination of diet digestibility in rabbits. *World Rabbit Science*, 3(1): 41-43. DOI: <https://doi.org/10.4995/wrs.1995.239>
- Pérez-Jiménez J, Neveu V, Vos F and Scalbert A (2010). Identification of the 100 richest dietary sources of polyphenols: an application of the phenol-explorer database. *European Journal of Clinical Nutrition*, 64(Suppl 3): S112-S120. Link: <https://www.nature.com/articles/ejcn2010221>
- Petrovic V, Marcincak S, Popelka P, Simkova J, Martonova M, Buleca J, Marcincakova D, Tuckova M, Molnar L and Kovac G (2012). The effect of supplementation of clove and agrimony or clove and lemon balm on growth performance, antioxidant status and selected indices of lipid profile of broiler chickens. *Journal of Animal Physiology and Animal Nutrition* 96: 970-977. DOI: <https://doi.org/10.1111/j.1439-0396.2011.01207.x>
- Prieto P, Pineda M and Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, 269(2): 337-341. DOI: <https://doi.org/10.1006/abio.1999.4019>
- Ragab MS (2012). Effects of using some natural feed additives on performance of growing Hy-line W-36 male chicks. *Egyptian J. Nutrition Feeds*, 15: 375-391. [Article Link](#)
- SAS (2004). *User's guide*. Statistic. SAS Inst. Cary, N.C. Raleigh. [Link](#)

- Schneider BH and Flatt WP (1975). The evaluation of feeds through digestibility experiments. University of Georgia Press, USA, pp. 423. Link: <https://lib.ugent.be/catalog/rug01:001983743>
- Shan B, Cai YZ Sun M and Corke H (2005). Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. Journal of Agricultural and Food Chemistry, 53(20): 7749-7759. DOI: <https://doi.org/10.1021/jf051513y>
- Shobana S and Naidu A (2000). Antioxidant activity of selected Indian spices. Prostaglandins, Leukotrienes and Essential fatty acids, 62(2):107-110 DOI: <https://doi.org/10.1054/plef.1999.0128>
- Silveira RF, Roque-Borda CA, Vicente EF (2021). Antimicrobial peptides as a feed additive alternative to animal production, food safety and public health implications: An overview. Animal Nutrition, 7(3):896-904. DOI: <https://doi.org/10.1016/j.aninu.2021.01.004>
- Srinivasan K (2005). Spices as influencers of body metabolism: An overview of three decades of research. Food Research International, 38(1): 77-86. DOI: <https://doi.org/10.1016/j.foodres.2004.09.001>
- Sulaiman A and Anas MS (2017). Quantitative determination of nutritional and anti-nutritional composition of clove (*Eugenia caryophyllata*). Journal of dairy and veterinary Science, 3(2):1-3 DOI: <https://doi.org/10.19080/JDVS.2017.03.555609>
- Suliaman AE, El Boshra Iman MO and El Khalifa EA (2007). Nutritive value of clove (*Syzygium aromaticum*) and detection of antimicrobial effect of its bud oil. Research Journal of Microbiology, 2(3): 266-271. Link: <https://scialert.net/abstract/?doi=jm.2007.266.271>
- Tariq H, Rao PVR, Raghuvanshi RS, Mondal BC and Singh SK (2015). Effect of Aloe vera and clove powder supplementation on carcass characteristics, composition and serum enzymes of Japanese quails. Veterinary World, 8(5):664-668. DOI: <https://doi.org/10.14202/vetworld.2015.664-668>
- Turgay O and Esen Y (2015). Antioxidant, total phenolic and antimicrobial characteristics of some species. Bulgarian Journal of Agricultural Science, 21(3): 498-503 Link: <https://www.agrojournal.org/21/03-05.pdf>
- Van der Zipp AJ, Frankena K, Boneschancher J and Nieuwland MGB (1983). Genetic analysis of primary and secondary immune responses in the chicken. Poultry Science, 62(4): 565-572. DOI: <https://doi.org/10.3382/ps.0620565>
- Windisch WM, Schedle K, Plietzner C, Kroismayr A (2008) Use of phytogetic products as feed additives for swine and poultry. Journal of Animal Science, 86(14 Suppl):140-148. DOI: <https://doi.org/10.2527/jas.2007-0459>
- Young DS (1997). Effects of drugs on clinical laboratory tests. Annuals of Clinical Biochemistry, 34: 579-581. DOI: <https://doi.org/10.1177/000456329703400601>