Influences of dietary herbal blend and feed restriction on growth, carcass characteristics and gut microbiota of growing rabbits

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ABSTRACT
The objective of the present study was to determine the effect of feed restriction systems, herbal mixture and their interactions on growth performance, carcass traits, and microbial aspects of growing New Zealand White (NZW) rabbit kept from 5 to 13 weeks of age. A 3×2 factorial arrangement was performed, including three feed restriction systems (ad-libitum, 90%, and 80% of ad-libitum) and four dietary supplementation levels of herbal mix (0, 0.30%, 0.50% and 0.70%). A total number of 120 rabbits (male and female ratio 1:1) at five weeks of age were randomly allotted into twelve experimental groups (n = 10 each). Results showed a significant decrease in body weight, body weight gain and feed intake in restricted-fed rabbits compared to the control group (ad-libitum). HERBS levels significantly influenced the growth performance and carcass traits of rabbits. The herbal blend had a positive effect on reducing the population of pathogenic microorganisms and increasing the population of lactic acid bacteria. Conclusively, it could be concluded that the feed restriction system has beneficial effects in the improvement of feed conversion ratio (FCR), weight gain, and carcass traits. In addition, HERBS supplementation to the growing rabbits resulted in significant improvements in growth performance, carcass characteristics, and microbial aspects of rabbits kept from 5 to 13 weeks of age.

HIGHLIGHTS:
- This work investigated the effect of feed restriction systems (FRS), herbal mix (HERBS), and their interactions with rabbits.
- Restricted feed decreased live body weight during all ages studied.
- Feed conversion ratio, weight gain and carcass traits were improved due to FRS.
- The HERBS improved the growth, carcass traits, and microbial aspects of rabbits.

Introduction
In rabbit production, feeding is the main cost, so feed intake control could adjust the diet and nutritional requirements to manage the growth performance (Yakubu et al. 2007; Bergaoui et al. 2008). In commercial farms, growing rabbits are usually fed ad-libitum (Maertens, 2009). After weaning in the rabbit, the early-life fast growth rate is accompanied by several problems, mainly high incidence of metabolic disorders and high mortality rate (Hassanabadi and Moghaddam 2006) and high incidence of skeletal diseases (Bovera et al. 2008).

In the growing rabbits, early feed restriction applied around post-weaning age could be of interest to improve feed efficiency (Tumova et al. 2002; Tumova et al. 2003; Yakibu et al. 2007; Gidenne et al. 2009; Gidenne et al. 2012), to induce compensatory growth (Tumova et al. 2002; Foubert et al. 2008), to reduce carcass fat deposition (Tumova et al. 2004), and to improve the digestibility of nutrients during the restricted feeding period (Tumova et al. 2004). Feed restriction suppresses growth during the restriction period, but the reduced growth can be compensated with greater future intake (Di Meo et al. 2007).
Digestive disorders are the leading cause of morbidity and mortality in growing rabbits, and are responsible for important economic losses in commercial rabbit farms (Ebeid et al. 2012). Therefore, early feed restriction could be a useful tool to improve biological and economic performance (Tumova et al. 2007), which is consequently involved in reducing production costs (Yakubu et al. 2007).

On the other hand, feeding can be restricted during the post-weaning period to improve feed efficiency and standardise growth curves in rabbits with different feed ingestion levels (Cavani et al. 1991) or to control the appearance of digestive disorders (Gidenne et al. 2009; Gidenne et al. 2011). The application of feed restriction during the fattening period of rabbits, without compromising too much the growth, maybe a good strategy for rabbit management because it may decrease the feeding cost and reduce the health risk (Foubert et al. 2008; Gidenne et al. 2012). Feed restriction during two and three weeks did not uniformly affect the carcass’s parts. Internal organs percentages increased with the increased length of feed restriction time respectively for 14 and 18 weeks, while the abdominal fat percentage significantly reduced to the length of time feed restriction at 14 weeks. (Sena et al. 2015).

Herbal extracts are potentially beneficial as growth promoters in diets and play a good role as therapeutic agents to treat certain diseases and disorders. They can replace antibiotics, enhancing immunity, and fight pathogenic bacteria and viral infections (Alimoh 2009). Beneficial impacts of herbs in poultry nutrition may include the enhancement of appetite and feed intake, the stimulation of endogenous digestive enzyme secretion, activation of immune response, antioxidant, antibacterial, and antiviral properties (Abd El-Hack et al. 2016).

Suganya et al. (2016) summarised that herbal extracts have a wide range of activities that stimulate feed intake and endogenous secretions and have antimicrobial, coccidiostats, or anthelmintic activity. A major field of herbal application is protecting animals and their products against oxidation. The presence of colonies of *Clostridium perfringens* and *Escherichia coli* in the colon content could be reduced by adding extracts of plants with capsaicin (1.98 g/100g) as with using of avilamycin in birds (Jamroz et al. 2003).

On the other hand, Alagawany et al. (2016) concluded that garlic supplementation (2, 4, and 6 g/kg) did not linearly and quadratically affect growth performance in rabbits while improving the immunity responses and lowered the lipid profile in blood and lipid peroxidation in liver and increased hepatic antioxidant activity in treated rabbits. Herbal mixture supplementation reduced plasma total cholesterol and triglycerides concentrations, whereas high-density lipoprotein HDL-cholesterol and glutathione peroxidase (GPX) were increased in broilers significantly. Furthermore, supplementation of the herbal mixture increased plasma levels of total protein and antibody titres for the Newcastle disease virus before and after the infection (Saleh et al. 2018).

Recently, Mossa et al. (2019) showed that medicinal plants are widespread in poultry feed as antibiotics alternatives. Several studies also confirmed the beneficial effects of phytogenic additives on growth performance, nutrient retention, gut health, intestinal microflora, reduced susceptibility to diseases, enhanced immunity function, and improved carcass yield and quality in poultry (Ashour et al. 2014; Ashour et al. 2020a, 2020b, 2020c).

Since feed restriction and the herbal mixture had positive impacts in growing rabbits, the objective of the present study was to investigate the impact of feed restriction, herbal mix and their interaction on the growth rate, carcass traits, several microbial aspects, and antioxidant activity of the growing rabbits, from 5 to 13 weeks of age.

**Materials and methods**

**Sample preparation**

*Capsicum annuum* (C.A.), *Pimpinella anisum* (P.A.), *Thymus vulgaris* (T.V.), *Mentha spicata* (M.S.), *Allium sativum* (A.S.), *Salvia rosmarinus* (S.R.), *Nigella sativa* (N.S.) were acquired from the local market at Zagazig city, Egypt. The herbs were cleaned then powdered. Individual herb was homogenised under two hours stirring with methanol 70% (1:10, w/v), then filtrated using Whatman No. 2. Methanol was restored at 45 °C in a BuCHI-B-480 rotary evaporator and then freeze-dried by Heto power dry LL 300 Freeze dryer and the obtained extracts were stored at 4 °C until use (Abd El-Hack et al. 2018; Saad et al. 2020).

**Microbial strains**

The gram-positive bacteria (*G*<sup>+</sup>) (*Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*) and the gram-negative bacteria (*G*<sup>−</sup>), (*Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella typhi*) besides, fungi (*Candida tropicalis*, *Candida albicans*, *Candida glabrata*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger*) were used to study the antimicrobial
activity of herbal mixture extract on these microorganisms in the current study.

**Antimicrobial and antifungal activity**

**Disc assay**

Antibacterial and antifungal activity of herbal mixture methanolic extract were estimated by disc diffusion method (Gulluce et al. 2007). The methanolic extract was dissolved in dimethyl sulfoxide (DMSO) to obtain (1–9 mg/mL) concentrations, then 100 μL of bacterial inoculum (1 × 10^8 CFU/mL) was spread on Mueller Hinton agar plates surface (MHA), and disc from fungal mycelium was seeded on the centre of potato dextrose agar (PDA) plates surface, then discs (6 mm) saturated with different concentrations of the herbal extract mixture solution and placed on both sides of MHA and PDA plates. Control was discs saturated with 10% DMSO. The plates were incubated at 37°C for 24 h (bacteria) and 28°C for 5 d (fungi) and observed the bacterial or fungal growth (Usman et al. 2014; El-Saadony et al. 2020).

**Synergistic effects**

The herbal extracts with higher antibacterial activity or lower MIC were selected, including methanolic extract of *Pimpinella anisum* (P.A.), *Thymus vulgaris* (T.V.), *Salvia rosmarinus* (S.R.), *Mentha spicata* (M.S.), and *Nigella sativa*. Five combinations of selected herbal extracts were tested against *Klebsiella pneumoniae* and *Staphylococcus aureus* (Nguefack et al. 2012).

**Animals and experimental design**

This experiment was carried out in the Rabbit farm of Animal and Poultry Production Department, Faculty of Technology and Development, Zagazig University, Zagazig, Egypt. All experimental procedures for the present study were conducted following the Local Experimental Animal Care Committee and subsequently approved by the Institutional Ethics Committee, Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt (ZU-IACUC/2/F/94/2018). The experimental period lasted for eight weeks, from September to October 2020. A 3 × 4 factorial arrangement was performed including three systems of feed restriction (*ad-libitum*, 90% and 80% of *ad-libitum*) and four dietary supplementations consisted of the basal diet as a control group and HERBS additives groups (3.0, 5.0 and 7.0 g HERBS/kg diet where a herbal mixture containing [(300 g garlic *Allium sativum* + 300 g *Capsicum annuum*) hot red pepper + 300 g *Thymus vulgaris* + 300 g *Salvia rosmarinus* + 150 g *Pimpinella anisum* + 150 g *Mentha spicata* + 300 g *Nigella sativa*) before that, these herbs were mixed and ground together in a powder form. A total number of 120 rabbits (male and female ratio 1:1) of weaning aged 5 weeks with 642.94 ± 13.68 g as a general mean of body weight were randomly divided into twelve experimental groups (*n* = 10 each) in five replicates each of two rabbits which housed in commercial wired cages (two animals per cage) with floor space of 0.15 m²/rabbit under 16 h photoperiod daily.

**Housing, feeding and environment**

Animals were housed in galvanised wired cages batteries (60 × 50 × 40 cm), in good natural ventilation through the windows (open housing system). Simultaneously, temperatures and humidity ranged...
between 22 to 35°C & 50 to 64% during experimental periods, respectively. The rabbits were fattened until 91 d of age (13 weeks). Cages were supplied with feeders and stainless steel nipples for feeding and drinking. Freshwater was available *ad-libitum*. Rabbits were examined daily for their healthy and clinically free from internal and external parasites, vaccinated against common diseases (inactivated clostridial vaccines at 6 weeks of age, inactivated viral haemorrhagic disease at 9 weeks of age, and inactivated *Pasteurella multocida* (bacterial haemorrhagic disease at 11 weeks of age) and kept under the same managerial and hygienic conditions.

The experimental diets were formulated to be iso-nitrogenous (16.54% CP) and iso-caloric (10.82 MJ DE/kg diet). Rabbits were fed pelleted commercial diets formulated to meet recommended nutrient requirements of rabbits, according to De Blas and Wiseman (2020), and the chemical composition of the basal diet was analysed according to Association Of Analytical Chemists (AOAC) (1995), as shown in Table 1. The Chemical characterisation of total phenolic, flavonoids and DPPH activity of solo or mix of herbal as shown in Table 2 (Ashour et al. 2020).

**Table 1.** Composition and chemical analysis of the experimental diet (as fed).

| Ingredient       | Basal diet |
|------------------|------------|
| Yellow corn      | 20.00      |
| Soybean meal 44% | 20.00      |
| Wheat bran       | 16.00      |
| Berseem hay      | 30.00      |
| Barley grain     | 10.00      |
| Molasses         | 2.00       |
| Limestone        | 1.00       |
| Salt             | 0.50       |
| Premix*          | 0.50       |
| Total            | 100.00     |

Analysed composition%**

| Analysed composition | Basal diet |
|----------------------|------------|
| Dry matter           | 88.06      |
| Organic matter       | 90.57      |
| Crude protein        | 16.54      |
| Ether extract        | 2.25       |
| Crude fibre          | 12.33      |
| Nitrogen free extract| 59.45      |
| Calcium              | 0.88       |
| Phosphorus           | 0.49       |
| Ash                  | 9.44       |
| Neutral detergent fibre| 32.00     |
| Acid detergent fibre | 18.10      |
| Acid detergent lignin| 4.00       |
| Starch               | 17.50      |
| Calcium/Phosphorus   | 1.79       |

Digestible energy (Kcal/kg) 2585

*Premix provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 1000 IU; vitamin E acetate, 50 mg; vitamin K3, 2 mg; biotin, 0.1 mg; thiamine, 2 mg; riboflavin, 4 mg; vitamin B6, 2 mg; vitamin B12, 0.1 mg; niacin, 40 mg; pantothenic acid, 12 mg; folic acid, 1 mg; choline chloride, 300 mg; iron, 100 mg; copper, 20 mg; magnesium, 50 mg; cobalt, 2 mg; iodine, 1 mg; zinc, 100 mg; selenium, 0.1 mg.

**Determined according to Association of Analytical Chemists (AOAC) (1995).

**Table 2.** Total phenolic (TP, mg GAE g⁻¹ extract) and total flavonoid (TF, mg QE g⁻¹ extract) contents and DPPH activity (SC₅₀, μg mL⁻¹) of the methanolic extract acquired from *Thymus vulgaris* (T.V.), *Pimpinella anisum* (P.A.), *Capsicum annuum* (C.A.), *Mentha spicata* (M.S.), *Salvia rosmarinus* (S.R.), *Allium sativum* (A.S.), *Nigella sativa* (N.S.) and its blend.

| Samples | TP, mg GAE g⁻¹ extract | TF, mg QE g⁻¹ extract | DPPH activity (SC₅₀, μg mL⁻¹) |
|---------|------------------------|----------------------|-------------------------------|
| T.V.    | 9.70                   | 2.40                 | 150                           |
| P.A.    | 12.15                  | 4.90                 | 100                           |
| C.A.    | 4.90                   | 0.90                 | 270                           |
| M.S.    | 10.23                  | 3.90                 | 118                           |
| S.R.    | 5.20                   | 1.80                 | 230                           |
| A.S.    | 6.60                   | 2.40                 | 200                           |
| N.S.    | 4.50                   | 1.30                 | 300                           |
| Blend   | 25                     | 16                   | 13                            |

*Measurements*

**Growth performance**

Initial body weight, body weight at 7, 9, 11 and 13 weeks of age, body weight gain, feed intake and feed conversion ratio were recorded. Also, the mortality rate was recorded during experimental periods. The weighing was carried out before offering the morning meal. Total body weight gain and feed conversion ratio were calculated biweekly, while feed conversion ratio (FCR) was calculated as feed to gain (g feed/g gain) ratio. Feed was supplied once per day (*ad-libitum*, 90% and 80% of *ad-libitum*).

**Carcass traits**

At the end of the experiment (13 weeks of age), five rabbits were randomly chosen for slaughtering from each group (one rabbit from each replicate) around the group’s mean after being fasted for 12 h. Slaughter procedures were carried out as described by Blasco and Ouhayoun (1996) by severing the carotid arteries and jugular veins, skinned, and eviscerated for measuring carcass parameters. Following the removal of the visceral organs and head, the remaining part of the carcass was weighed as carcass weight, which was then expressed as a percentage of the fasted weight to obtain the dressing percentage. as follow: Dressing % = (hot carcass weight/fasted weight) x 100. The carcass was separated into three cuts: (1) fore part
(including thoracic insertion muscles), (2) mid part (including the abdominal wall and the ribs after the 7th thoracic rib), and (3) hind part (including the sacral bone and the lumbar vertebra after the 6th lumbar vertebra). The relative weights of the liver, kidneys, and heart (Giblets) were determined using the formula:

\[
\text{Giblets weight, \%} = \frac{\text{Giblets weight (wt)}/\text{Pre-slaughter weight of rabbit} \times 100}{\text{Dressing weight, \%}} = \frac{\text{Carcass weight} + \text{giblets weight}/\text{Pre-slaughterwt} \times 100}{\text{Slaughter weight of rabbit}}
\]

**Microbial count in diet and caecal samples**

The feed samples were microbiologically examined at an interval of 0, 2, 4, 6, and 8 weeks. Dietary samples were mixed with sterile saline peptone solution 1:10 (w/v) at the screw bottle and homogenised for three min. Different media were used to calculate microorganisms. Total bacterial count (TBC) was counted at plate count agar at 30°C for 2 d. The total yeasts and moulds count (TYMC) were estimated on Rose Bengal Chloramphenicol agar for five days at 25°C. Total coliforms were counted on violet red bile agar after 24 h incubation at 37°C (Harrigen and Mccance-Margart 1976). *Escherichia coli* was counted on eosin methylene blue agar plates after incubation for 24 h at 37°C (Oxoid 1982). All plates were examined for typical colony types and morphological characteristics associated with each culture medium. On the other hand, the microbial counts in rabbit caecum were estimated as in diet. Caecal samples (five-replicate) were homogenised in a screw bottle with sterilised saline peptone solution (1:10, w/v). Decimal serial dilutions up to 10^7 were prepared. The different microorganisms were enumerated as Kurtzman and Fell (1984). MRS-medium was used to count Lactic acid bacteria, according to Argyri et al. (2013). *Enterococcus* spp. was counted in *Chromocultfl enterococci* agar (Miranda et al. 2005).

**Statistical analysis**

Data were statistically analysed using SPSS (2014) according to Sendcor and Cochran (1982) as the following model:

\[
Y_{ijk} = \mu + A_i + S_j + AS_{ij} + e_{ijk}
\]

Where: \(Y_{ijk}\) = an observation, \(\mu\) = the overall mean, \(A_i\) = effect of restricted grades (i=ad-libitum, 90% and 80% to 3), \(S_j\) = effect of herbal mixture level (j=0, 0.30, 0.50 and 0.70%), \(AS_{ij}\) = the interaction between restricted grades and herbal mixture level and \(e_{ijk}\) = random error.

Data of the effect of herbal mixture on the caecal bacterial count and bacterial count reduction in the diet were analysed using one-way ANOVA using SPSS (2014).

Data presented as means ± S.E. Means were considered significant at \(p > .05\). According to Duncan’s multiple tests, significant differences between treatment means were tested (Duncan 1955).

**Results**

**Antimicrobial activity of herbal mixture extract**

The antibacterial effect of the methanolic herbal mixture on pathogenic bacteria is shown in Table 3. G–ve bacteria more resistant to herbal extract than G+ve bacteria. The inhibition zones diameter (IZD) significantly (\(p \leq .05\)) increased with the increment of herbal mixture extract concentration. *Staphylococcus aureus* is a more sensitive G+ve bacteria to the herbal mixture (0.9 mg/mL) with IZD 33.7 mm with a relative increase of about 20% over *Bacillus cereus* and 25% above *L. monocytogenes*. On the other hand, *Klebsiella pneumonia* more susceptible than *Escherichia coli* and *Salmonella typhi*, with a relative increase of about 5 and 10%, respectively. MIC expressed the least concentration inhabit the bacterial population, but MBC was the lowest concentration of herbal mixture kills the bacteria. Herbal mixture extract MIC was higher against *Listeria monocytogenes* with 0.8 mg/mL followed by *B. cereus* and *S. aureus*. On the level of G– bacteria *Salmonella typhi* needs a high level of the herbal mixture with 0.95 mg/mL than other G– bacteria. MBC ensures the bacterial kill. The bacteria were ordered in descending, *S. typhi*, *E. coli*, *K. pneumonia*, *L. monocytogenes*, *B. cereus*, and *S. aureus* according
to MBC. Table 3 showed considerable antifungal activity against *C. tropicalis*, *C. albicans*, *C. glabrata*, *A. flavus*, *A. fumigatus* and *A. niger*. The highest herbal methanolic extract MIC and MFC were against *C. tropicalis* followed by *A. niger* with 0.99/1.90 and 0.9/1.75 mg/ml.

The herbal mixture of thyme, anise, mint, rosemary, and black seed showed the highest antibacterial methanolic extract with inhibition zones ranged (19–25 mm), (14–18 mm) for *S. aureus* and *K. pneumonia*, respectively besides, the highest synergistic effect on *S. aureus* and *K. pneumonia* (Table 4).

### Growth performance

Results in Table 5 showed a significant decrease in live body weight of growing rabbits with restriction feed during all ages studied (7, 9, 11 and 13 weeks). On the contrary, HERBS addition significantly increased rabbits’ body weight at the level of 0.50% (2211 g/animal) at the end of the experiment (13 weeks). From the view of the interaction, there was a significant influence due to the main factors studied. The highest body weight was obtained by ad-libitum followed by 90% and 80% of ad-libitum, respectively. The highest body weight under ad-libitum (FRS)/C3 HERBS (50%) with an average of 2197 g/animal. On the other hand, daily weight gain decreased significantly with restriction feed during all experimental periods except 11–13 weeks of age (as shown in Table 6). Bodyweight gain significantly increased due to HERBS addition and the interaction effect at all interval periods. The best values were recorded with HERBS levels of 0.50 or 0.70% under different FRS. The mortality rate was not shown because there was no mortality detected during the experimental period; that is why the feed restriction system, HERBS, and their interaction did not affect the growing rabbit’s mortality rates.

### Table 3. Antimicrobial activity of herbal mixture against pathogenic bacteria and fungi as shown by the inhibition zone diameter (mm).

| Microorganisms | Items | Herbal mixture concentration, mg/100g | 100 | 300 | 500 | 700 | 900 | MIC | MBC |
|----------------|-------|---------------------------------------|-----|-----|-----|-----|-----|-----|-----|
| **G + bacteria** |       |                                       |     |     |     |     |     |     |     |
| *B. cereus*     |       |                                       | 9 ± 0.3b | 12.6 ± 0.6b | 17.5 ± 0.5b | 24.0 ± 0.5bb | 28.7 ± 0.4b | 60d | 110d |
| *S. aureus*     |       |                                       | 11.4 ± 0.2a | 15.6 ± 0.5a | 23.8 ± 0.2a | 27.4 ± 0.3a | 33.7 ± 0.2a | 50e | 95e  |
| *L. monocytogenes* |     |                                       | 8.5 ± 0.4b | 12.4 ± 0.6b | 18.4 ± 0.4b | 22.5 ± 0.4c | 27.5 ± 0.5b | 80c | 150c |
| **G – bacteria** |       |                                       |     |     |     |     |     |     |     |
| *E. coli*       |       |                                       | 7.7 ± 0.5c | 10.4 ± 0.7cd | 14.7 ± 0.3c | 19.8 ± 0.2d | 24.7 ± 0.3c | 89b | 175ab|
| *S. typhi*      |       |                                       | 7.2 ± 0.5cd | 9.7 ± 0.7d | 14.2 ± 0.5c | 16.6 ± 0.3e | 23.4 ± 0.4cd | 95a | 180a |
| *K. pneumoniae* |       |                                       | 7.8 ± 0.5c | 11.8 ± 0.8c | 15.3 ± 0.6d | 15.5 ± 0.4ef | 22.3 ± 0.2d | 92ab | 165b |
| **Fungi**       |       |                                       |     |     |     |     |     |     |     |
| *C. tropicalis* |       |                                       | 7.9 ± 0.4c | 9.9 ± 0.2cd | 11.8 ± 0.2de | 14.2 ± 0.4e | 16.9 ± 0.2d | 99a | 190a |
| *C. albicans*   |       |                                       | 7.8 ± 0.5c | 9.2 ± 0.4cd | 12.5 ± 0.3d | 15.3 ± 0.5d | 18.9 ± 0.3c | 87c | 165c |
| *C. glabrata*   |       |                                       | 8.9 ± 0.3b | 10.2 ± 0.2c | 13.4 ± 0.4cd | 15.7 ± 0.2cd | 19.9 ± 0.2b | 85c | 161cd|
| *A. flavus*     |       |                                       | 9.9 ± 0.2a | 12.4 ± 0.3a | 16.8 ± 0.5a | 19.5 ± 0.3a | 21.9 ± 0.4a | 75d | 140d |
| *A. fumigatus*  |       |                                       | 7.7 ± 0.6d | 11.3 ± 0.1b | 15.7 ± 0.2ab | 17.8 ± 0.5b | 19.5 ± 0.6b | 82cd | 170b |
| *A. niger*      |       |                                       | 6.6 ± 0.5e | 10.4 ± 0.4c | 14.4 ± 0.3c | 16.9 ± 0.4c | 18.2 ± 0.2cd | 90b | 175b |

The values are the mean ± SE. Values in the same column with different letters are significantly different (p < .05), n = 3.

### Table 4. Synergetic effect of higher methanolic extract combination as shown by the inhibition zone diameter (mm).

| Methanolic extract (900 mg/100g) | Staphylococcus aureus | Klebsiella pneumoniae |
|---------------------------------|-----------------------|-----------------------|
| Thyme                           | 24ab                  | 18a                   |
| Anise                           | 21bc                  | 17ab                  |
| Mint                            | 23b                   | 17ab                  |
| Rosemary                        | 25a                   | 16b                   |
| Hot papper                      | 19cd                  | 15bc                  |
| Garlic                          | 19cd                  | 14c                   |
| Black seed                      | 20c                   | 18a                   |
| Extract combination             |                       |                       |
| Thyme, mint, rosemary, anise    | 28b                   | 20b                   |
| Thyme, mint, rosemary           | 29ab                  | 21ab                  |
| Anise, rosemary, black seed     | 23cd                  | 18c                   |
| Thyme, mint, rosemary, black seed | 25c                | 19bc                  |
| Thyme, mint, rosemary, anise, black seed | 30a            | 22a                   |

The values are the mean ± SE. Values in the same column with different letters are significantly different (p < .05), n = 3.

The results of Table 3 showed considerable antifungal activity against *C. tropicalis*, *C. albicans*, *C. glabrata*, *A. flavus*, *A. fumigatus* and *A. niger*. The highest herbal methanolic extract MIC and MFC were against *C. tropicalis* followed by *A. niger* with 0.99/1.90 and 0.9/1.75 mg/ml.

The herbal mixture of thyme, anise, mint, rosemary, and black seed showed the highest antibacterial methanolic extract with inhibition zones ranged (19–25 mm), (14–18 mm) for *S. aureus* and *K. pneumonia*, respectively besides, the highest synergistic effect on *S. aureus* and *K. pneumonia* (Table 4).
Table 5. The effect of FRS, HERBS and their interaction on body weight of growing rabbits.

| Items                  | 5w | 7w | 9w | 11w | 13w |
|------------------------|----|----|----|-----|-----|
| **Body weight, g**     |    |    |    |     |     |
| **Main effect**        |    |    |    |     |     |
| Feed restriction system (FRS) |    |    |    |     |     |
| Ad-libitum             |    |    |    |     |     |
| 90%                    | 647| 898| 1295|1657a|2183a|
| 80%                    | 645| 788b|1153b|1576b|2072b|
| SEM                    | 4.16|9.37|11.06|13.02|12.10|
| p-value                | 0.138|0.012|0.001|0.003|0.010|
| Herbal percentage (HERBS, %) |    |    |    |     |     |
| 0                      | 646|817b|1172c|1551c|2059bc|
| 0.300                  | 644|829b|1281b|1613b|2108b|
| 0.500                  | 642|867a|1352a|1734a|2211a|
| 0.700                  | 639|863a|1350a|1737a|2198a|
| SEM                    | 4.80|6.01|9.33|10.50|13.12|
| p-value                | 0.738|0.031|0.010|0.007|0.001|
| Interaction (FRS* HERBS) |    |    |    |     |     |
| FRS                    | HERBS, % |    |    |     |     |
| 0                      | 642|857|1233c|1604c|2121b|
| 0.300                  | 642|863|1288b|1635bc|2145ab|
| 0.500                  | 648|882|1322a|1695a|2197a|
| 0.700                  | 656|880|1322a|1697a|2190a|
| 0.500                  | 656|802|1162d|1563d|2065d|
| 0.300                  | 644|808|1217c|1594c|2090c|
| 0.500                  | 649|827|1252bc|1655b|2141ab|
| 0.700                  | 633|825|1251bc|1657b|2135ab|
| 0.300                  | 640|791|1115e|1490f|2055e|
| 0.500                  | 647|797|1170d|1521e|2029de|
| 0.700                  | 628|816|1205cd|1581cd|2061c|
| 0.300                  | 627|814|1204cd|1583cd|2074c|
| 0.700                  | 627|814|1204cd|1583cd|2074c|
| SEM                    | 13.680|14.840|14.070|15.550|17.020|
| p-value                | 0.252|0.084|0.023|0.007|0.011|

Means in the same column within each effect without common superscripts are different at the level. p < .05; W: weeks.

Table 6. The effect of FRS, HERBS and their interaction on body weight gain of growing rabbits.

| Items                  | 5–7w | 7–9w | 9–11w | 11–13w | 13–15w |
|------------------------|-------|------|-------|--------|--------|
| **Body weight gain, g** |       |      |       |        |        |
| **Feed restriction system (FRS)** |       |      |       |        |        |
| Ad-libitum             |       |      |       |        |        |
| 90%                    | 251a  | 397a | 362b  | 526    | 1536a  |
| 80%                    | 143b  | 365b | 423a  | 496    | 1427b  |
| SEM                    | 119b  | 324c | 405b  | 522    | 1315c  |
| p-value                | 0.010 | 0.041| 0.009 | 0.053  | 0.007  |
| Herbal mixture percentage (HERBS, %) |       |      |       |        |        |
| 0                      | 171c  | 355c | 379a  | 508a   | 1413c  |
| 0.300                  | 185b  | 425b | 322b  | 495a   | 1464b  |
| 0.500                  | 225a  | 485a | 382a  | 477b   | 1569a  |
| 0.700                  | 224a  | 487a | 387a  | 461c   | 1559a  |
| SEM                    | 5.66  | 7.05 | 6.32  | 8.10   | 11.28  |
| p-value                | 0.010 | 0.001| 0.008 | 0.114  | <0.001 |
| Interaction (FRS* HERBS) |       |      |       |        |        |
| FRS                    | HERBS% |      |       |        |        |
| (Ad-libitum)           | 0     |      |       |        |        |
| 0                      | 211ab | 376b | 370b  | 517    | 1474bc |
| 0.300                  | 218ab | 424b | 347c  | 510    | 1500b  |
| 0.500                  | 238a  | 441a | 372b  | 501    | 1552a  |
| 0.700                  | 237a  | 442a | 374b  | 493    | 1547a  |
| (90%)                  | 0     |      |       |        |        |
| 0                      | 157c  | 360de| 401a  | 502    | 1420d  |
| 0.300                  | 146c  | 408c | 377b  | 495    | 1445cd |
| 0.500                  | 184b  | 425b | 402a  | 486    | 1499b  |
| 0.700                  | 183b  | 426b | 405a  | 478    | 1493b  |
| (80%)                  | 0     |      |       |        |        |
| 0                      | 150c  | 398a | 359c  | 515    | 1364a  |
| 0.300                  | 157c  | 398a | 356d  | 508    | 1389a  |
| 0.500                  | 177b  | 404c | 361c  | 499    | 1442cd |
| 0.700                  | 176b  | 405c | 363c  | 491    | 1437cd |
| SEM                    | 6.34  | 8.10 | 7.64  | 15.47  | 14.09  |
| p-value                | 0.023 | 0.001| 0.008 | 0.114  | <0.001 |

Means in the same column within each effect without common superscripts are different at the level. p < .05; W: weeks.

**Feed intake and feed conversion ratio**

Results in Table 7 given a significant decrease in feed intake of growing rabbits due to RFS (90 or 80%) during all ages studied 5–13 weeks of age by (10.16% and 20.14%, respectively) compared to an ad-libitum group. In contrast, HERBS insignificantly influenced feed intake of growing rabbits at all experimental terms studied. Simultaneously, the interaction effect presented significant effects due to the main factors studied and the highest feed intake recorded at all levels and HERBS under the ad-libitum feed restriction systems studied. The feed conversion ratio (FCR) was significantly (p < .01) improved for growing rabbits with feed restriction system (FRS) (90 or 80%) as compared to ad-libitum ones during all intervals studied, as reported in Table 8. Also, the dietary addition of HERBS significantly (p < .01) improved FCR of growing rabbits at the level of 0.50% and 0.70%, which recorded 4.06 and 4.08 respectively, at the whole experiment (5–13w). The interactions between FRS and dietary HERBS significantly (p < .05) affected on FCR values at 5–7w and 7–9w intervals, while insignificantly influenced the other experimental periods (Table 8).
Carcass traits

Results in Table 9 illustrated the significant increase in dressing %, hindquarter % and giblets % due to restriction feed of 90 or 80% as compared to ad-libitum ones, on contrary gastrointestinal % increased significantly with ad-libitum feeding as compared with other FRS (90 or 80%). The remaining carcass traits (forequarter % and lion %) were insignificantly affected due to FRS. On the other hand, HERBS addition was insignificantly influenced all carcass traits studied (Table 9). Furthermore, the interaction effect on all carcass traits examined was insignificant, except for gastrointestinal %, which increased significantly with high levels of HERBS addition (0.5% or 0.7%) under ad-libitum feeding, as shown in Table 9.

Microbial count in diet and caecum

The total bacterial (TBC), total yeasts and moulds (TYMC), coliform, and E. coli counts in rabbit diet supplemented with different concentrations of the herbal mixture (0.3, 0.5, 0.7%) after eight weeks are presented in Table 10. The results indicated that the diet supplemented with herbal mixture 0.7% significantly decreased TBC by 30% after eight weeks compared to the diet supplemented with 0.3%. The effect of FRS, HERBS and their interaction on a feed conversion ratio of growing rabbits.

| Items                  | Forequarter | Loin | Hindquarter | Gastrointestinal | Giblets | Dressing |
|------------------------|-------------|------|-------------|------------------|---------|----------|
| Feed restriction system (FRS) |             |      |             |                  |         |          |
| Ad-libitum             | 34.52       | 29.27| 36.25b      | 15.41a           | 2.34bc  | 56.35b   |
| 90%                    | 33.84       | 29.04| 37.12a      | 14.94b           | 2.56b   | 57.94a   |
| 80%                    | 34.15       | 28.90| 36.93a      | 14.45c           | 2.91a   | 58.17a   |
| SEM                    | 2.08        | 1.02 | 2.71        | 1.03             | 0.15    | 0.22     |
| p-value                | <0.001      | 0.001| <0.001      | <0.001           | 0.041   | 0.006    |

Herbal mixture A percentage (HERBS, %)

| Items                  | Forequarter | Loin | Hindquarter | Gastrointestinal | Giblets | Dressing |
|------------------------|-------------|------|-------------|------------------|---------|----------|
| 0                      | 34.26       | 29.01| 36.71       | 14.89c           | 2.43    | 57.32    |
| 0.300                  | 33.64       | 28.70| 37.35       | 15.12b           | 2.41    | 57.76    |
| 0.500                  | 34.51       | 28.87| 36.62       | 15.29a           | 2.38    | 57.60    |
| 0.700                  | 34.38       | 29.13| 36.45       | 15.32a           | 2.29    | 57.63    |
| SEM                    | 2.73        | 1.49 | 3.04        | 0.95             | 0.28    | 3.11     |
| p-value                | 0.10        | 0.04 | 0.04        | 0.04             | 0.091   | 0.018    |

Table 9. The effect of FRS, HERBS and their interaction on carcass traits of growing rabbits.
control group. Besides, TYMC, coliform, and *E. coli* count decreased by 20%, 50%, and 15%, respectively.

A significant decrease in the caecal microbial count was observed with the herbal mixture supplementation at different concentrations (0.3, 0.5, and 0.7%), but a considerable increase in lactic bacteria count (Table 11). The diet reduction from *ad-libitum* to 90% of diet decreased bacterial count from log 9.96 to 9.66. Besides, 80% diet +0.7% herbal mixture significantly decrease about 15% of the control diet. *Salmonella* did not detect in 80% diet +0.7% herbal mixture and lactic count significantly increased from log 3.5 to 4.8. The phenolic compounds and essential oil in the herbal mixture positively affected microbial count reduction.

### Discussion

**Antimicrobial activity of herbal mixture extract**

The presence of antimicrobial secondary metabolites in the tested herbal mixture includes phenolic compounds (flavonoids and anthocyanidins), phenolic acids (hydroxybenzoic) terpenoids, alkaloids, glycosides, saponins, lectins, steroids, and polypeptides (Kumar et al. 2008; Janisan et al. 2009; Ganesan, Xu. 2018) is the possible reason for the microbiocidal effect of herbal mixture extract. These compounds can eliminate microbes through many mechanisms like membrane permeability, capsules formation, affecting cell membranes’ metabolism and formation, preventing bacterial biofilm and reducing the production of bacterial toxins. The antimicrobial activity of herbal mixture also recorded by Berektsi et al. (2018) who revealed that the inhibition zone of some methanolic plant extracts ranged from 6 to 23 mm against the tested bacteria *S. aureus, E. faecalis, E. cloacae*. While MICs ranged from 0.1 to 12.8 mg/mL. *P. Vulgaris, C. monspeliensis*, and *P. granatum* methanolic extracts showed higher activity against the tested bacteria than the other extracts. Besides, Masoumian and Zandi (2017) screened the antimicrobial activities of myrtle’s alcoholic extract and cinnamon’s water extract compared to penicillin against tested microorganisms. The distance of inhibition in the range of 23—28 mm, and the width of penicillin’s inhibition zone against *Staphylococcus aureus* was larger than the plant extracts. However, MIC of myrtle extract with 30 mg/

### Table 10. Effect of herbal mix with different levels on the caecal bacterial count.

| Samples | Total count bacteria | Total yeasts and moulds | *E. coli* | *Salmonella* spp. | Enterococcus spp. | Coliform | Lactic acid bacteria |
|---------|----------------------|------------------------|----------|-------------------|-------------------|----------|--------------------|
| Control | 9.96 ± 0.5a          | 4.79 ± 0.2a            | 5.76 ± 0.8a | 2.84 ± 0.2a       | 6.83 ± 0.3a       | 6.79 ± 0.2a | 3.53 ± 0.2 cd       |
| 0.30%   | 9.45 ± 0.4b          | 4.65 ± 0.3b            | 5.37 ± 0.8b | 1.87 ± 0.1b       | 6.67 ± 0.4b       | 6.52 ± 0.4b | 3.66 ± 0.5c         |
| 0.50%   | 9.13 ± 0.7bc         | 4.34 ± 0.5c            | 5.12 ± 0.9c | 1.11 ± 0.2c       | 6.34 ± 0.7c       | 6.21 ± 0.2bc | 3.89 ± 0.4f         |
| 0.70%   | 8.76 ± 0.5c          | 3.87 ± 0.6d            | 4.78 ± 0.5d | ND                | 5.64 ± 0.2d       | 5.59 ± 0.8cd  | 4.13 ± 0.2ef        |
| 90%     | 9.66 ± 0.4ab         | 4.72 ± 0.1a            | 5.69 ± 0.6ab | 2.52 ± 0.3ab      | 6.77 ± 0.4ab      | 6.74 ± 0.7d  | 4.24 ± 0.3e         |
| 90% + 0.3% | 9.39 ± 0.2b         | 4.51 ± 0.2b            | 5.23 ± 0.2b | 1.06 ± 0.2c       | 6.54 ± 0.2bc      | 6.46 ± 0.2b  | 4.36 ± 0.4de        |
| 90% + 0.5% | 8.86 ± 0.3c         | 4.14 ± 0.5c            | 4.92 ± 0.3c | ND                | 6.12 ± 0.3c       | 5.96 ± 0.3c  | 4.47 ± 0.1d         |
| 90% + 0.7% | 8.59 ± 0.1c         | 3.63 ± 0.6d            | 4.61 ± 0.4d | ND                | 5.51 ± 0.4d       | 5.41 ± 0.4d  | 4.51 ± 0.2cd        |
| 80%     | 9.61 ± 0.2ab         | 4.66 ± 0.2ab           | 5.51 ± 0.2b | 2.41 ± 0.5ab      | 6.69 ± 0.8b       | 6.66 ± 0.5b  | 4.66 ± 0.1c         |
| 80% + 0.3% | 9.31 ± 0.5b         | 4.47 ± 0.3b            | 5.16 ± 0.1bc | ND                | 6.48 ± 0.3bc      | 6.58 ± 0.4b  | 4.73 ± 0.2b         |
| 80% + 0.5% | 8.75 ± 0.3c         | 4.07 ± 0.3c            | 4.85 ± 0.2c | ND                | 6.09 ± 0.8c       | 5.86 ± 0.6c  | 4.77 ± 0.3ab        |
| 80% + 0.7% | 8.51 ± 0.9c         | 3.99 ± 0.7d            | 4.54 ± 0.3d | ND                | 5.47 ± 0.4d       | 5.36 ± 0.2d  | 4.83 ± 0.2a         |

ND: not detected, the values mean ± SE, n = 3, means in the same column with different lowercase letters indicate significant differences between treatments.
mL showed considerable antibacterial activity compared to the other extracts and even penicillin. Also, Atef et al. (2019) studied the effect of different extracts of *Moringa oleifera* L. (leaves) and *Matricaria recutita* L. (flowers) against 12 resistant MDR, XDR, and PDR test isolates; they found that methanolic and aqueous extract revealed good antibacterial activity overall isolates, whereas ethanolic extract showed the lowest activity, with MIC ranging from 7.8–62.5 mg/mL. Moreover, Gonelimali et al. (2018) studied the antimicrobial activity of some herbal ethanolic extracts against tested bacterial isolates; they found that rose-lle extract had significant antibacterial activity against all tested bacteria except *Candida albicans* (CA). But clove and thyme extracts showed antifungal effects against CA with inhibition zones in the range of 15.8–25.2 mm. Furthermore, Dellavalle et al. (2011) evaluated acidic, aqueous, and buffer extracts of medicinal plants against *Alternaria spp*. The MIC values were between 1.25 and 25 μg/mL, while MFC was 1.25 μg/mL for rosemary and 10 μg (*Cynara scolymus* L.). They also found that the values of MICs and MFCs of (*Salvia officinalis* L. and *R. officinalis* leaves and seed extracts of (*Salvia sclarea* L.) are very similar to the commercial fungicide Captan (2.5 g/mL). Therefore, these plant extracts are considered potential antifungal agents against fungal diseases in the plant.

The Mixing of herbs maximised the antimicrobial activity of bioactive compounds’ e.g. phenolic compounds (flavonoids, coumarins), tannins, and thiosulfenates lucosinolates, and saponins. The obtained results in this study were similar to Negi (2012), who observed that methanolic plant extracts had antimicrobial and synergistic activity because of the presence of phenolic compounds. Also, Masoumian and Zandi (2017) found that the herbal mixture of Aloevera, myrtle, and henna was more effective against *P. aeruginosa* and *S. aureus*; the width of reduction zones was 15 and 21 mm, respectively. However, each herb’s reduction zones in the mixture were in the range of 17–28 mm on *S. aureus* culture and lower zones on *P. aeruginosa* culture. The antagonist effect on the tested bacteria population because of active compounds exist in the herbal mixture. Al-bayati (2008) studied the synergistic antibacterial effects of methanolic extracts and essential oils of clove and anisum on nine bacterial strains and found the inhibitory effects of the methanolic extracts and essential oils combination on most of the tested bacteria. The extract used in this study showed consistent effect with the inhibitory impacts of extracts used by Al-bayati (2008) on tested bacteria.

### Growth performance

This noticeable improvement in live body weight (LBW) at the whole experimental period (5–13 weeks of age) might be due to improved digestion and absorption of diet nutrients by some components of the phytogenic additives (Tables 2 and 5). However, that may be contributed to enhancing the utilisation of feed consequence, enhancing the growth rate. Prohibition of using antibiotics in poultry production due to herbs and plant medicines as feed additives to improve growth condition, its induced saliva secretion and improve digestion processes (Suganya et al. 2016; Ashour et al. 2020d, Menchetti et al. 2020). Phytagenic additives may also reduce the environmental problems produced by using antibiotics as feed additives, such as bacterial resistance (Peric et al., 2009). Also, Ahmed et al. (2002) pointed out that live body weight and daily weight gain were improved significantly by adding garlic powder to the rabbit diet.

Also, Onu and Aja (2011) reported that garlic supplementation by 0.25% produced significant (*p* < .05) effects on weight gain and significantly enhanced the hematological parameters of rabbits as well. The mortality rate detected during the experimental period showed no differences between all groups due to the main factors studied or the interaction, while it agreed with Foubert et al. (2008), Ebeid et al. (2012) and Abou-Kassem (2017). They indicated that feed restriction did not influence the mortality percentage of growing rabbits. Gidenne et al. (2012) showed that a more long restriction (for 2 or 3 weeks) of growing rabbits reduced mortality and morbidity from digestive troubles. While in contrast, El-Speiy et al. (2015) reported that feed restriction significantly decreased the mortality percentage in growing rabbits compared with the *ad-libitum* ones. Present results agreed with Ashour et al. (2020d) and Castrica et al. (2020), who found a significant difference (*p* < .05) in live body weight and weight gain due to the addition of the herbal mixture powder at all studied periods.

Present results agreed with Abou-Kassem (2017), who showed a significant (*p* < .01) increase in feed intake of growing rabbits with *ad-libitum* group (control) as compared with other restriction ones during all experimental periods studied (5–13 weeks of age). These results in line with Alabiso et al. (2017) summarised that the restriction feed system for a 3-week post-weaning of growing rabbits significantly decreased feed intake by (−22 to −24 g dry matter/day) and gave a lower feed conversion ratio than *ad-libitum* feeding. On the other hand, results disagreed with Di Meo et al. (2007), who studied the effects of...
ad-libitum and feeding restricted for growing rabbits from 5 to 12 weeks of age and observed no difference in the bodyweight daily weight gain results.

The dietary inclusion of Foeniculum vulgare Mill. Seeds with oregano leaves have been reported to improve feed conversion ratio. Moreover, the dietary inclusion of a mixture of Trigonella foenum-graecum L., Cassia senna L., and Lupinus albus L. played an important role as a growth promotor in rabbits (Dalle Zotte et al. 2016). Ahmed et al. (2002) pointed out that the feed conversion ratio was improved significantly by adding garlic powder to the rabbit diet. Additionally, Onu and Aja (2011) reported that garlic supplementation by 0.25% produced significant ($p < .05$) effects on feed intake and feed conversion ratio and also it significantly enhanced the hematological parameters of rabbits as well. Recently, Ashour et al. (2020d) reported that the best FCR recorded in chicks fed the diet supplemented with herbal mixture powder at a level of 5.0 g/kg diet, while the worst one recorded by the group fed level of 3.0 g/kg diet during the total term, 0–5 week old in broilers.

**Carcass traits**

Our results agree with findings by Abou-Kassem (2017), who found that dressing weight % significantly increased by feed restriction compared to ad-libitum feed for rabbits. Present results showed that gastrointestinal % increased with ad-libitum feed system as compared to other FRS. These results disagreed with Abou-Kassem (2017), who indicated that empty gastrointestinal tract on pre-slaughter live weight was significantly ($p < .01$) higher for restriction than ad-libitum feed of growing rabbit.

Regarding giblets %, our findings agreed with those found by Abou-Kassem (2017), who clarified that liver weight % significantly ($p < .001$) increased with restriction feed system as compared to ad-libitum group. Also, Alabiso et al. (2017) found that a 3-week feed restriction after weaning have a heavier carcass produced by growing rabbits fed ad-libitum ($+100$ g; $p < .001$), and they added that production of lighter carcasses could be compensated partly by the lower feed conversion ratio gave by restricted rabbits. While these results disagreed with those obtained by Yakubu et al. (2007), who studied the effects of feed restriction on weaned rabbits and reported no difference in the liver % compared with ad-libitum group. Also, El-Speiy et al. (2015) found that fed different restriction strategies had insignificant effects on some relative organs weight of growing rabbits. No studies have been reported on the interaction between the FRS and HERBS, which contained several herbal plants as in this investigation.

**Microbial count in diet and caecum**

These natural microbicidal are divided into two types of phytoanticpins that inhibit microbes and phytoalexins, which are antioxidants that induce an immune response (Sukalingam et al. 2018). Cushnie et al. (2007), El-Adawi, (2012) and Awolola et al. (2014) revealed that secondary metabolites are divided into three main groups: phenolic compounds, terpenes, and alkaloids. The mechanics of action of these compounds in removing bacteria is through the destruction of the cell membrane, the production of ROS, the prevention of the formation of biofilms, the building of cellular cells, and the proliferation of microbial DNA ATP. Besides, by synergizing with antibiotics, these compounds increase the killing of disease-causing microbes. Kone et al. (2016) studied the effect of dietary supplementation with cranberry, onion, and strawberry extracts and essential oils on the bacterial count in weaned rabbit meat; the results indicated that the dietary supplementation with polyphenolic extracts and essential oils has a significant ($p < .05$) positive effect in reducing bacterial load against the control group.

In conclusion, no adverse effects on performance and meat quality traits were observed with the diet-supported plant extracts and essential oils. Still, the effect can be optimised by investigating higher doses. Pogány et al. (2020) showed a reduction in coliforms counts, clostridia, and staphylococci count, but lactic acid count increased in the intestine of broiler rabbits. Namkung et al. (2004) studied the effect of herbal extracts and organic acids supplementation to feed for four weeks on weaned pigs (growth performance, gut microbiota and immune response). The animals were divided into groups: the control group, 1.1% acid group 1 containing (acetic, propionic, phosphoric and citric acid) and acid two groups with 1% (acid + lactic acid) and the herbal group (cinnamon, thyme and oregano) by 0.75% addition and an Antibiotic group (Lincomycin 110 ppm). The first and second groups showed the highest ADG only during the second week, while the herbal extract had the lowest ADG during the third week only. The number of coliforms was lowest on day 4 in the first and second groups, while on day 14 in the herbal extract and antibiotics groups. The number of lactobacilli decreased in the antibiotic group after two weeks. Based on PCR-DGGE,
the additives affected the numbers of gut microbes, as antibiotics’ addition reduced both coliform and lactobacilli. In contrast, the herbal extracts and acids supplementation to the dietary system only reduced coliforms. Mixtures of organic acids and herbal extracts can supplement the feed as an alternative to antibiotics for the first weeks.

Conclusions
It could be concluded that the feed restriction system improved feed utilisation by giving the best values of FCR. It also has a beneficial effect on improving weight gain, feed efficiency, and carcass traits. Also, HERBS dietary addition had great improvement effects on growth performance, carcass traits and microbial aspects of growing rabbits kept from 5 to 13 weeks of age. Based on the positive experimental effects obtained on rabbit’s health and production, further studies are needed to maximising these positive effects with specific herbal formulations.

Author contributions
Conceptualisation and methodology, D.E.A.K., K.M.M., R.A.E.-S., M.T.E.-S., K.A.E.T, M.E.A.E.-H., A.E.T and E. A.S. Data curation, M.E.A.E.-H., M.E., A.E.T, and E.A.S. Writing—original draft preparation, D.E.A.K., M.T.E.-S., K.A.E.T and M.E.A.E.-H. Writing—review and editing, M.E.A.E.-H., M.E.S., M.E., K.M.M., A.E.T. and K.A.E.T. All authors have read and agreed to the version of the manuscript.

Ethical Approval
All experimental procedures for the present study were conducted following the Local Experimental Animal Care Committee and subsequently approved by the Institutional Ethics Committee, Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt (ZU-IACUC/2/F/94/2018).

Disclosure statement
No potential conflict of interest was reported by the author(s).

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