

## Effect of herbal extracts as alternatives to antibiotics in the first week of age on broiler performance, serum biochemistry, and intestinal morphology under commercial farm conditions

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### Abstract

This study was performed to show the effect of two herbal extracts as alternatives to antibiotics in the first eight days of rearing on broiler performance, serum biochemistry, and the jejunum morphology of the broiler. A total of 264, one-day Ross 308 chicks were used. They were randomly distributed into four treatments including three replicates of each. T1 was the control (nothing in drinking water), T2 had antibiotics in the drinking water (1 g/L of Gentadox), T3 had ENTERIA in the drinking water (0.5ml/L), T4 had ICEN in the drinking water (0.5ml/L). These products were administered for the first eight consecutive days of rearing. In the first seven days, there was no substantial variation in performance found in all groups. From days 8–14, T3 markedly improved body weight gain (BWG). The feed conversion ratio (FCR), thyroid hormones, total protein, and globulin were substantially improved in T3 and T4. The edible internal organs and heart percentage was substantially increased in the herbal groups. All the jejunum histology parameters, except the crypt depth, were substantially increased in T3. The T3 recorded the best bodyweight uniformity and economic efficiency compared to other groups. Similarly, the yolk sac residual at days 4 and 7 was lower in this group compared to other groups. It can be concluded that herbal extracts can replace antibiotics in improving and enhancing general broiler performance.

**Keywords:** chicken, ENTERIA, FCR, histology, ICEN, phytogetic plant

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### Introduction

Since the implementation of the restriction on the utilization of antibiotics as growth stimulants in animal feed, the enhancement of the immune function and antioxidant defence in poultry has been a hot topic. Alternative food components that support animal growth without having unfavourable side effects (such as pathogenic strains developing antibiotic resistance) are in high demand. Numerous studies have examined the positive effects of probiotics, prebiotics, acidifiers, enzymes, and herbs on the intestinal microbiota of monogastric animals. Numerous active components found in herbs and spices can have a variety of effects on animals, including antibacterial, immunomodulatory, and antioxidant effects. As a result, they may have an impact on both the productivity and health of animals in addition to the grade of animal products (Madhupriya *et al.*, 2018; Gürsoy, 2021; Nobakth *et al.*, 2022). ICEN is composed of *Harpagophytum procumbens*, *Laurus nobilis*, *Origanum heracleoticum*, and *Ribes nigrum* and ENTERIA contains *Citrus paradisi*, *Olea europaea*, and *Punica granatum*.

Pomegranate (*Punica granatum* L.) is a fruit from the Punicaceae family which has been utilized for several therapeutic purposes in traditional medicine for millennia (Ajaikumar *et al.*, 2005). The antibacterial efficacy of *P. granatum* L. peel and seed extract against a variety of bacteria and fungi has been demonstrated in various *in vitro* experiments (Dahham *et al.*, 2010; Nuamsetti *et al.*, 2012; Shiga 2012). In addition, Ahmed & Yang (2017) demonstrated that *P. granatum* by-product linearly increased the serum IgA and IgG in broiler and therefore immunity was improved. Yamasaki *et al.*

(2006) reported the immunomodulatory effects of *P. granatum* L. seed oil, which is high in punicic acid, in mice.

High amounts of phenols, flavonoids, and vitamins, particularly vitamin C, can be found in grapefruit peels (Manthey 2004; Vlaicu *et al.*, 2020). Red blood cell levels of antioxidant enzymes are increased by vitamin C and polyphenols (Hasin *et al.*, 2006). Citrus fruits have been demonstrated in prior research to be useful in lowering blood cholesterol levels (Parmar & Kar 2008). According to Oluremi *et al.* (2006), orange waste (~15%) had a good effect on the growth performance in the production of broilers. Due to a large number of phenolic components, olive byproducts like olive cake and other phytogetic leaves or extracts (such as *Moringa oleifera*) have also frequently been used in poultry and have substantially improved growth performance, meat quality, and health status (Saleh *et al.*, 2014, 2020; Saleh and Alzawqari, 2021; Selim *et al.*, 2021).

According to estimates, *Laurus nobilis* contains 1–3% eugenol, in addition to a variety of other active compounds, such as monoterpenes, cinnamaldehyde, thymol, and carvacrol. Because it contains many phenolic and antimicrobial chemicals, it also has antioxidant properties (Erturk *et al.*, 2006). Comparing the laurel leaf treatment to the control treatment, lower levels of malondialdehyde were found in the serum of treated quail. Quail given laurel leaves showed observable improvements in several productive features, including egg output, egg weight, and cholesterol and triglyceride levels (Karaalp & Genc, 2013). Thymol and carvacrol are the two primary components of oregano essential oil (OEO), which is produced from plants belonging to the *Origanum* genus (Oniga *et al.*, 2018). OEO has received more attention in recent years as a result of its positive benefits on preserving gut health. However, there is a significant discrepancy in the studies of how OEO affects the performance of chickens. According to several studies, oregano supplements or dried oregano leaves had no impact on animal performance or gastrointestinal traits (Bampidis *et al.*, 2005; Barreto *et al.*, 2008; Cerisuelo *et al.*, 2014). However, Peng *et al.* (2016) discovered that OEO consumption had a favourable impact on intestinal health, which in turn enhanced broiler production performance and carcass attributes. Blackcurrant syrup is a rich supply of polyphenols (1.91 g/L), and studies have shown that it contains greater than three times more antioxidant activity than apple or orange juice (Piljac-Zegarac *et al.*, 2009). Approximately 245 mg/g of polyphenolic extracts from blackcurrant fruit, of which 100 mg/g are anthocyanins, are present in blackcurrant juice (Denev *et al.*, 2010). Because most of the applied rearing programs for broilers include the use of antibiotics in the first week of broiler production, this study was performed to investigate the effect of two commercial products (Enteria and ICEN) which are composed of the above-mentioned compounds as an alternative to antibiotics on broiler performance, intestinal histology, and some serum biochemical parameters.

## Materials and Methods

The ethical clearance for this research was granted by the Animal Ethics Committee at the Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok, Iraq (Ethical Clearance number: AEC 5102022)

For this research, a total of 264, day-old chicks (Ross 308) with initial body weights ( $41.05 \pm 0.013$  g to  $41.40 \pm 0.062$  g) were used. The initial weight of each replicate was measured on the first day, and the chicks were divided evenly and randomly among the various treatments: T1, control (no additive in drinking water); T2, adding Gentadox (each kg 100g gentamycin 100g doxycycline), 1 g/L for 8 consecutive days; T3, adding Enteria in drinking water (0.5 mL/L) for 8 consecutive days; and T4 adding ICEN in drinking water (0.5 mL/L) for 8 consecutive days.

The herbal extract products (ICEN and ENTERIA) were provided by Biodevas Laboratories in France. Each treatment consisted of three replicates with 22 chicks in each. For all phases of the trial, raising took place in floor pens. The raising halls were furnished with the necessary equipment, such as heating systems and programmable thermostats. The chicks were given an *ad libitum* diet along with a treatment diet for 1–21 d. Every week, the live body weight was recorded. The following equation was used to compute the weight gain:

$$\text{WG (g)} = \text{final weight} - \text{initial weight} \quad (1)$$

The following formulae were used to compute feed intake and feed conversion ratio:

$$\text{FI/birds (g)} = (\text{feed introduced} - \text{residual feed}) / \text{number of birds} \quad (2)$$

$$\text{FCR} = \text{feed intake (g)} / \text{weight gain (g)} \quad (3)$$

The following formula was used to compute mortality:

$$\text{Mortality (\%)} = \frac{\text{the number of (dead chicks / total chicks)} \times 100}{1} \quad (4)$$

The diet which was used in this experiment was obtained from Bade Company, Iraq (Table 1).

**Table 1** Ingredients of the diet used in the experiment

Ingredient	kg/ton	
	Starter 1–14 days	Grower 15–21 days
Yellow Corn	312.25	332.25
Wheat	200	250
Wheat bran	70	50
Soya bean meal (46%)	350	300
Vegetable Oil	20	22
Limestone	2	1
Di-calcium phosphate	2	1
DL-methionine	1	1
Lysine	1	1
Anti-toxin	1	1
Anti-coccidiostat	0.250	0.250
Salt	0.500	0.500
Premix <sup>1</sup>	40	40
Formulated chemical composition		
Crude protein (%)	22.70	20.84
Energy (ME/kg diet)	2915	3010
Fat %	4.03	4.27
Linoleic acid %	1.92	2.03
Crude fibre %	3.22	2.92
Methionine %	0.62	0.60
Lysine %	1.55	1.41
Tryptophan %	0.37	0.34
Met + cystine %	0.74	0.69
Arginine %	1.58	1.43
Threonine %	1.00	0.93
Calcium %	1.27	1.02
Available phosphorus %	0.63	0.57
Sodium %	0.21	0.19

<sup>1</sup> calcium 26%, available phosphorus 8%, sodium 3.5%, lysine 3%, methionine 2%, threonine 1.5%; additives per kg: Vitamin A 270000 IU, Vit D3 80000 IU, Vit E 1000 mg, Vit K3 40 mg, Vit B1 60 mg, Vit B2 180 mg, Vit B3 1000mg, Vit B5 250 mg, Vit B6 40 mg, Vit B9 30 mg, Vit B12 0.3 mg, Vit H2 3 mg, choline 9000 mg, Se 4 mg, copper 250 mg, iodine, 18 mg, iron 600 mg, manganese, 1800 mg, zinc 2500 mg

At 1, 4, and 7 days of age, two chicks were randomly chosen from each replicate. After killing, the yolk sac was measured as a percentage of live body weight. Throughout the experiment, individual birds in each replicate were weighed once a week to estimate the body weight uniformity:

$$\text{Body weight uniformity \%} = \frac{\text{number of chicks within mean} \pm 5\%}{\text{total number of chicks}} \quad (5)$$

Two birds were randomly chosen from each replication and killed with a sharp blade at the end of the study (which lasted 21 days). Following the removal of feathers, the following internal organ percentages were determined:

$$\text{Internal organ (\%)} = \left( \frac{\text{organ weight}}{\text{live body weight}} \right) \times 100 \quad (6)$$

Two chickens from each replication were selected at 21 days old to provide the jejunum tissue sample. The tissues were preserved in 10% formalin after being properly washed with normal saline. Haematoxylin and eosin were used to stain the tissue. The tissue samples were then fixed to the

plates using paraffin wax. Following the method of Iji *et al.* (2001), a 10× light microscope (Dino-Eye-Microscope Eyepiece 38 digital Camera) was used to measure the desired parameters.

Economic efficiency (cost–benefit ratio) was calculated according to the method of Mustafa (2022). Data were subjected to analysis of variance (ANOVA) in a general linear model using IBM SPSS Statistics v26. When significant treatment effects were detected at a probability level of  $P < 0.05$ , Duncan's test was applied to determine the statistical differences between means.

## Results and Discussion

The effect of herbal extracts and antibiotics on broiler performance during 21 days of rearing is shown in Table 2. During the first 7 d of rearing, there was no effect of treatments on the studied variables except that FCR was improved ( $P < 0.05$ ) in the group of chicks that had 0.5 ml/L water of Enteria product in the drinking water. Ali and Al-Shuhaib (2021) found no effect of adding laurel to the chicken ration on chicken body weight in the first week of rearing. At 8–14 days of rearing, chicks in the ENTERIA group (T4) had a higher body weight (BW) than the other groups ( $P < 0.05$ ). Similar results were found by Omar *et al.* (2016); higher weights and FCRs were found in chickens that had herbal extracts in the drinking water. Zangana *et al.*, (2022) stated that the addition of herbal extract to chicken drinking water substantially improved the FCR and BW. Murray *et al.* (1991) stated that because some herbs included a combination of necessary fatty acids, including linolenic and linoleic acids, they may have a good impact on body weight and general performance. Although the feed intake and daily feed intake were non-significant among T1, T3, and T4, they were better ( $P < 0.01$ ) better than the group in which the antibiotic was used (T2). The FCR was improved ( $P < 0.01$ ) in herbal groups compared to the control and antibiotic groups. It is possible that feeding herbal extract improved the small intestine's ability to digest protein, which would explain the improvement in the FCR (EL-Gendi, 1996). In general, and during the entire experiment, ICEN was substantially better than Enteria (0.5 ml/L of water) in improving BW, BWG, and daily weight gain, and both of them substantially increased these parameters compared to the other two treatments. The FCR was substantially improved by the addition of herbals to the drinking water over that of the other two groups. According to Toghyani *et al.* (2010), broiler BW and FCR are substantially impacted by a modest dose (5 g/kg) of thyme. Moreover, in Najafi *et al.* (2010), the group that had a diet that included thyme had noticeably superior body weight and feed conversion ratio. Liveability was not affected in all groups and these results were previously found by Zangana *et al.*, (2022). However, these results disagree with those found by Omar *et al.*, (2016) who noticed a decrease in mortality in the chicken group that had herbal extract in their drinking water.

Table 3 shows the influence of herbal extract as an alternative to antibiotics on broiler serum biochemical tests. Although there were no marked differences between the control group and herbal groups in uric acid, creatine kinase, glucose, and AST, they were substantially improved compared to the antibiotic group. These results concur in terms of glucose level with those found by Abd El-Hady *et al.*, (2020). However, they found no marked effect of herbal supplementation on the uric acid level. Moreover, the ALT was better in the herbal groups compared to control and antibiotic groups ( $P < 0.001$ ). Abd El-Hady *et al.* (2020) found similar results when they included the mixed essential oils in the chicken drinking water. They concluded that the levels of ALT and AST were likely to indicate that the liver was being protected from hepatic degeneration. The liver contains the enzymes ALT and AST, which it releases into the blood when it is damaged (Sherwin, 2003). The thyroid hormones, T3 and T4, were higher in the ICEN group than the Enteria group and both of them were better than the control and antibiotic groups ( $P < 0.01$ ). Hassan *et al.* (2016) claimed similar results when they fed broilers different levels of *Moringa oleifera* meal. The thyroid hormones are crucial for protein synthesis and growth (Hayashi, 1993). As a result, growth retardation results from decreased protein turnover, which in turn results from decreased T3 synthesis. Thyroid hormone levels rose when ICEN or Enteria products were added to drinking water. According to Hassan *et al.*, (2016), this ultimately led to an increase in plasma total protein concentration, followed by protein synthesis, which improved broiler productivity (increased live body weight and body weight gain), explaining the findings of the current study. Total protein and globulin were increased ( $P < 0.05$ ) in chicks that had herbal extract in the drinking water. This would suggest that the broilers had a stronger immune system. It is hypothesized that a bird's plasma protein profile will reflect the metabolic processes involved in protein production and/or breakdown. It is known that stressful situations can cause the adrenal gland cortex to secrete corticosterone, which substantially increases protein catabolism due to its gluconeogenic action (Tollba, 2003).

**Table 2** Effect of herbal extracts and antibiotics on broiler performance during various rearing periods

Variables	Treatments				P-value
	T1	T2	T3	T4	
<b>1–7 days</b>					
Day old chick weight (g)	41.05±0.013	41.08±0.081	41.40±0.062	41.07 ±0.014	0.371
Body weight (g)	200.00 ± 1.52	203.70 ± 1.85	202.01± 1.03	204.01 ± 0.75	0.214
Weight gain (g)	158.94 ± 1.50	162.62 ± 1.88	160.61 ± 0.86	162.94 ± 0.59	0.191
Daily weight gain (g)	22.70 ± 0.21	23.23 ± 0.26	22.94 ± 0.12	23.27 ± 0.08	0.191
Feed intake (g)	154.38 ± 1.75	155.74 ± 2.87	149.91 ± 0.087	149.89 ± 0.17	0.083
Daily feed intake (g)	22.05 ± 0.25	22.24 ± 0.41	21.42 ± 0.012	21.41 ± 0.025	0.083
Feed conversion ratio (kg/kg)	0.971 ± 0.019 a	0.957 ± 0.006 ab	0.933 ± 0.005 bc	0.920 ± 0.004 c	0.039
Liveability (%)	100 ± 0.00	96.30 ± 3.20	100 ± 0.00	100 ± 0.00	0.052
<b>8–14 days</b>					
Body weight (g)	559.64 ± 3.16 b	562.79± 3.76b	576.49 ± 3.96ab	582.55 ± 9.26 a	0.048
Weight gain (g)	359.64 ± 7.59	359.08 ±3.31	374.47 ± 8.51	378.53 ± 16.93	0.107
Daily weight gain (g)	51.37 ± 1.08	51.29 ± 0.47	53.49 ± 1.21	54.07 ± 2.41	0.107
Feed intake (g)	452.63± 2.63 b	467.44 ± 8.27 a	447.36± 3.23 b	450.87 4.01 b	0.004
Daily feed intake (g)	64.66 ± 0.37b	66.77 ± 1.18a	63.90 ± 0.24 b	64.41 ± 0.57 b	0.004
Feed conversion ratio (kg/kg)	1.258 ± 0.02a	1.301 ± 0.01 a	1.195 ± 0.02b	1.193 ± 0.05b	0.008
Liveability (%)	98.25 ± 3.03	100 ± 0.0	100 ± 0.0	96.48 ± 3.04	0.219
<b>15–21 days</b>					
Body weight (g)	908.15 ± 3.03 c	920.68 ± 4.24b	994.95 ± 3.06a	985.69 ± 0.93a	0.0001
Weight gain (g)	348.50 ± 6.13 b	357.88 ± 2.46 b	418.45 ± 6.90a	403.13 ± 9.19 a	0.0001
Daily weight gain (g)	49.78 ± 0.87 b	51.12 ± 0.35 b	59.77 ± 0.98 a	57.57 ± 1.31 a	0.0001
Feed intake (g)	607.01 ± 9.15	614.86± 9.07	612.86 ± 10.23	628.70 ± 2.44	0.364
Daily feed intake (g)	86.71 ± 1.30	87.83 ± 1.29	87.55 ± 1.46	89.81 ± 0.34	0.364
Feed conversion ratio (kg/kg)	1.742 ±0.027 a	1.718± 0.031 a	1.465 ± 0.025 b	1.561 ±0.041b	0.001
Liveability (%)	98.33± 1.66	96.67 ± 1.65	98.33 ± 1.64	96.67 ± 1.67	0.802
<b>1–21 days</b>					
Day old chick weight (g)	41.05 ± 0.013	41.08 ± 0.081	41.40 ± 0.062	41.07 ± 0.014	0.371
Body weight (g)	908.15 ± 3.30 c	920.68± 1.80c	994.95 ± 3.06a	985.69±0.93 b	0.0001
Weight gain (g)	867.09 ± 3.04 c	873.09 ± 1.74 c	953.54 ± 2.90a	944.61± 0.94 b	0.0001
Daily weight gain (g)	41.29 ± 0.144 c	41.57 ± 0.082 c	45.40 ± 0.138 a	44.98± 0.044 b	0.0001
Feed intake (g)	1214.03± 7.49	1238.05 ± 16.54	1210.14 ± 10.27	1229.49 ± 2.54	0.275
Daily feed intake (g)	57.81 ± 0.35	58.95± 0.78	57.62 ± 0.48	58.54 ± 0.12	0.275
Feed conversion ratio (kg/kg)	1.400 ± 0.009 a	1.418 ± 0.019 a	1.269 ± 0.009 b	1.301 ± 0.002 b	0.0001
Liveability (%)	98.33 ± 1.66	96.67 ± 1.68	98.33 ± 1.65	96.67 ± 1.67	0.802

T1 was the control (nothing in drinking water), T2 had antibiotics in the drinking water (1 g/L of Gentadox), T3 had ENTERIA in the drinking water (0.5ml/L), T4 had ICEN in the drinking water (0.5ml/L)

**Table 3** Broiler serum biochemical parameters under the effect of herbal extracts and antibiotics

Variables	Treatments				P-value
	T1	T2	T3	T4	
Uric acid (mg/dl)	2.50 ± 0.08 <sup>b</sup>	3.65 ± 0.20 <sup>a</sup>	2.63 ± 0.11 <sup>b</sup>	2.61 ± 0.09 <sup>b</sup>	0.0001
Creatine kinase (U/L)	2413.17 ± 142.8 <sup>b</sup>	3269.50 ± 98.01 <sup>a</sup>	2218.33 ± 55.15 <sup>bc</sup>	2109.33 ± 35.24 <sup>c</sup>	0.0001
Glucose (mg/dl)	184.50 ± 2.5 <sup>b</sup>	198.50 ± 2.9 <sup>a</sup>	188.83 ± 3.6 <sup>b</sup>	184.33 ± 3.2 <sup>b</sup>	0.013
AST (U/L)	187.00 ± 2.9 <sup>b</sup>	252.50 ± 9.1 <sup>a</sup>	182.33 ± 1.9 <sup>b</sup>	188.35 ± 3.1 <sup>b</sup>	0.0001
ALT (U/L)	2.59 ± 0.09 <sup>b</sup>	3.20 ± 0.03 <sup>a</sup>	2.23 ± 0.03 <sup>c</sup>	2.35 ± 0.06 <sup>c</sup>	0.0001
T3 (nmol/ml)	2.36 ± 0.04 <sup>c</sup>	2.34 ± 0.09 <sup>c</sup>	3.20 ± 0.06 <sup>a</sup>	2.80 ± 0.07 <sup>b</sup>	0.0001
T4 (nmol/ml)	3.95 ± 0.10 <sup>c</sup>	3.51 ± 0.16 <sup>c</sup>	4.60 ± 0.14 <sup>a</sup>	3.24 ± 0.08 <sup>b</sup>	0.0001
T3/T4	0.600 ± 0.018 <sup>c</sup>	0.670 ± 0.017 <sup>bc</sup>	0.702 ± 0.035 <sup>b</sup>	0.867 ± 0.032 <sup>a</sup>	0.0001
Total protein (mg/dl)	2.10 ± 0.03 <sup>b</sup>	2.32 ± 0.23 <sup>b</sup>	2.77 ± 0.13 <sup>a</sup>	2.86 ± 0.09 <sup>a</sup>	0.003
Albumin (mg/dl)	1.05 ± 0.031	0.995 ± 0.037	1.015 ± 0.021	1.024 ± 0.018	0.456
Globulin (mg/dl)	1.040 ± 0.066 <sup>b</sup>	1.333 ± 0.20 <sup>b</sup>	1.760 ± 0.13 <sup>a</sup>	1.835 ± 0.078 <sup>a</sup>	0.001

T1 was the control (nothing in drinking water), T2 had antibiotics in the drinking water (1 g/L of Gentadox), T3 had ENTERIA in the drinking water (0.5ml/L), T4 had ICEN in the drinking water (0.5ml/L)

The effect of herbal extracts as alternatives to antibiotics on the internal organs of broilers reared in commercial farm conditions is shown in Table 4. Edible internal organs were not affected by treatments compared to the control group, except that the heart percentage was decreased ( $P < 0.05$ ) in groups that had herbal extracts in their drinking water, compared to the control and antibiotic groups. These results concur with Zaker-Estighamati *et al.* (2021), who claimed a marked decrease in the heart in chickens that a mixture of two herbal plants in their diet at the rate of 500 × 500 mg/kg. However, Sadeghi *et al.* (2012), Hassan *et al.* (2016), and Sigolo *et al.* (2021) used different herbal plants in their experiments without any statistical differences in the percentage or weight of the heart or other inner edible parts. The current results disagree with those of Sigolo *et al.* (2021) in that they found a marked influence of herbal plants on the spleen, liver, and bursa weights. The length of the small intestine increased ( $P < 0.05$ ) in T3 compared to all other treated groups. This was similar to cecal length, which increased ( $P < 0.005$ ) in both groups treated with herbal extract. Toghyani *et al.* (2010) used an antibiotic and a herbal plant and they found that both of them substantially increased the intestinal length compared to the control group. Similarly, Sadeghi *et al.* (2012) found a marked increase in the intestine length of broilers when administered with a herbal extract.

**Table 4** The effect of herbal extracts and antibiotics on the internal organs of broiler chickens (% of live weight) reared on commercial farms

Variables	Treatments				P-value
	T1	T2	T3	T4	
Liver %	3.19 ± 0.17	3.01 ± 0.10	3.62 ± 0.13	3.60 ± 0.27	0.072
Spleen %	0.057 ± 0.008	0.066 ± 0.013	0.077 ± 0.004	0.070 ± 0.008	0.506
Gizzard %	1.33 ± 0.055	1.44 ± 0.073	1.51 ± 0.038	1.40 ± 0.045	0.141
Bursa %	0.186 ± 0.02	0.225 ± 0.03	0.192 ± 0.01	0.186 ± 0.01	0.618
Heart %	0.539 ± 0.04 <sup>a</sup>	0.540 ± 0.02 <sup>a</sup>	0.427 ± 0.02 <sup>b</sup>	0.432 ± 0.02 <sup>b</sup>	0.015
Small intestine %	5.36 ± 0.32	5.51 ± 0.13	5.46 ± 0.11	5.51 ± 0.25	0.955
Small intestine length (cm)	131.83 ± 3.02 <sup>b</sup>	134.86 ± 2.62 <sup>b</sup>	144.67 ± 1.28 <sup>a</sup>	137.67 ± 2.02 <sup>b</sup>	0.006
Cecal length (cm)	10.71 ± 0.42 <sup>b</sup>	10.41 ± 0.27 <sup>b</sup>	13.66 ± 0.71 <sup>a</sup>	12.48 ± 0.73 <sup>a</sup>	0.002

T1 was the control (nothing in drinking water), T2 had antibiotics in the drinking water (1 g/L of Gentadox), T3 had ENTERIA in the drinking water (0.5ml/L), T4 had ICEN in the drinking water (0.5ml/L)

The jejunum histology of chickens under the effect of antibiotics and herbal extracts is shown in Table 5. All the measured parameters differed substantially under the studied treatments. Villi height, villi apical width, villi base width, villi height to crypt depth ratio, and surface area were substantially higher in the chicken group that had ENTERIA in the drinking water. However, crypt depth was substantially lower in this group. Our results are in agreement with those of Ding *et al.* (2020), in which they obtained higher villi height and lower crypt depth of the jejunum in ducks fed herbal extracts. Another study by Gul *et al.* (2019) stated that the administration of 600 g/kg of oregano essential oil to laying hens increased villus height. Increased villus height and villus height/crypt depth in the jejunum may improve the amount of absorbable material as well as the effectiveness of digestion and absorption (Mohammadi *et al.*, 2014). Furthermore, according to Windisch *et al.* (2008), the antioxidant properties of herbal essential oils are what cause an improvement in intestinal morphology. However, these results disagreed with those claimed by Ali and Al-Shuhaib (2021), who found an increase in crypt depth in the jejunum of broilers that were fed laurel leaves.

**Table 5** Intestinal histology of broiler chickens fed herbal extracts and antibiotics

Variables	Treatments				P-value
	T1	T2	T3	T4	
Villi height (µm)	464.49± 7.35 <sup>d</sup>	495.53± 8.58 <sup>c</sup>	660.18± 12.5 <sup>a</sup>	571.70 ± 11.2 <sup>b</sup>	0.0001
Crypt depth (µm)	154.64 ± 5.58 <sup>a</sup>	151.38± 3.43 <sup>ab</sup>	129.54 ± 6.67 <sup>c</sup>	137.5± 6.65 <sup>bc</sup>	0.007
Villi apical width (µm)	64.65± 1.52 <sup>c</sup>	89.55± 2.17 <sup>d</sup>	107.25± 0.76 <sup>a</sup>	97.75± 0.79 <sup>b</sup>	0.0001
Villi base width (µm)	95.11 ± 0.84 <sup>c</sup>	98.11± 1.1 <sup>d</sup>	117.44± 0.56 <sup>a</sup>	100.45± 0.57 <sup>b</sup>	0.0001
Villi height/crypt depth	3.122 ± 0.12 <sup>c</sup>	3.317± 0.08 <sup>c</sup>	5.420 ± 0.24 <sup>a</sup>	4.403 ± 0.19 <sup>b</sup>	0.0001
Surface area (µm <sup>2</sup> )	1159.02± 25.3 <sup>b</sup>	1047.91± 21.5 <sup>c</sup>	1384.10 ± 26.8 <sup>a</sup>	1160.14± 23.3 <sup>b</sup>	0.0001

T1 was the control (nothing in drinking water), T2 had antibiotics in the drinking water (1 g/L of Gentadox), T3 had ENTERIA in the drinking water (0.5ml/L), T4 had ICEN in the drinking water (0.5ml/L)

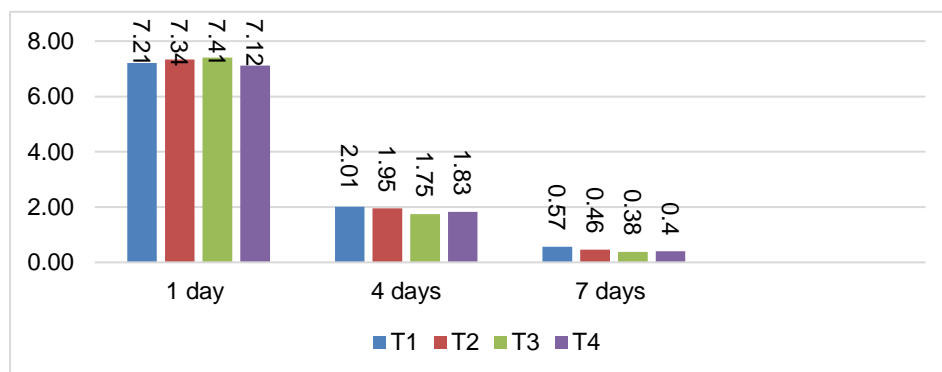
The difference between the two herbal extracts and antibiotics and their effect on economic efficiency in broilers raised under commercial farm conditions is shown in Table 6. The cost of feed was reduced when ICEN was used, followed by Enteria; Gentadox recorded the highest cost of feed per kg. Although there was no difference among all treatments in the price of live body weight (kg), the total cost of body weight (kg) was less in the ICEN treatment compared to Enteria and both of them were lower than the Gentadox treatment. Similarly, the ICEN treatment recorded the best cost-to-benefit ratio followed by the Enteria and Gentadox treatments, respectively. The current results are in agreement with the results found by Omar *et al.* (2016), who found a 13% improvement in the group of chickens given herbal extracts in their drinking water. Similarly, Zangana *et al.*, (2022) found a 14.05% improvement in the economic efficiency of a broiler group which had herbal plant extracts in the drinking water.

**Table 6** The economic efficiency of chicken administered two herbal extracts as alternatives to antibiotics

Items	T2	T3	T4
Feed cost per kg	0.73045	0.713983	0.725399
Gentadox cost	0.173	-----	
Enteria	-----	0.0482	
ICEN	-----	-----	0.0715
Total cost/kg BW	0.90345	0.762183	0.796899
Price/kg LBW	1.9	1.9	1.9
Profit/kg	0.996551	1.137817	1.103101
Cost–benefit ratio	1.103051	1.492841	1.384242
Improvement %		38.97904	28.1191

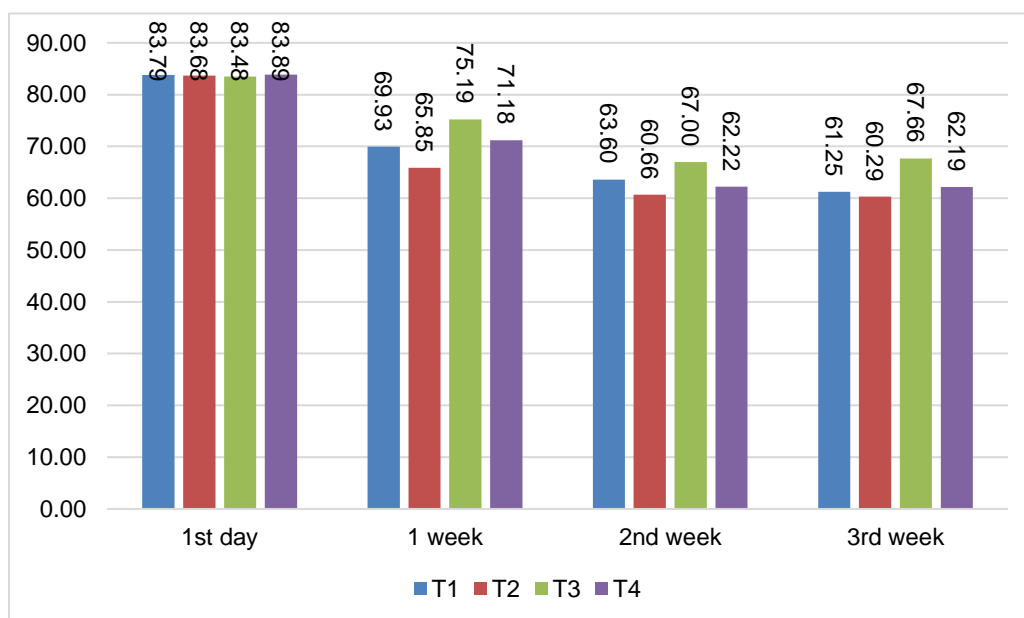
T1 was the control (nothing in drinking water), T2 had antibiotics in the drinking water (1 g/L of Gentadox), T3 had ENTERIA in the drinking water (0.5ml/L), T4 had ICEN in the drinking water (0.5ml/L)

The percentage of yolk sac to live body weight in all treated groups is shown in Figure 1. For the three periods (1, 4, and 7 d), there was no marked difference among all the treated groups. However, the ENTERIA group (T3), followed by the ICEN group (T4), recorded the lowest yolk sac percentage to live body weight compared to other groups in the 4th and 7th days of rearing. The current results agree with those of Iji *et al.* (2001) in which the yolk sac was 8% of chick weight upon hatching and by day 7, was <1% of the BW.



**Figure 1** Effect of using herbal extract as an alternative to antibiotics on residual yolk sac  
 T1 was the control (nothing in drinking water), T2 had antibiotics in the drinking water (1 g/L of Gentadox), T3 had ENTERIA in the drinking water (0.5ml/L), T4 had ICEN in the drinking water (0.5ml/L)

Body weight uniformity at different periods of rearing in broiler chickens fed herbal extracts as alternatives to antibiotics is illustrated in Figure 2. On the first day, the uniformity percentage was almost the same among all treated groups. Later, in the 1st, 2nd, and 3rd weeks of age, the third group had the best and highest uniformity percentage compared to the other groups. In the second group in which the antibiotic was used, the uniformity percentage was lowest from the first week to the end of the experimental period. An improvement in the jejunum parameters that is claimed in this study may be the reason for better nutrient utilization in the groups which had herbal extracts.



**Figure 2** Impact of using herbal extracts as alternatives to antibiotics on body weight uniformity (%) of broiler chicks at different ages  
 T1 was the control (nothing in drinking water), T2 had antibiotics in the drinking water (1 g/L of Gentadox), T3 had ENTERIA in the drinking water (0.5ml/L), T4 had ICEN in the drinking water (0.5ml/L)

**Conclusion**

It can be concluded that herbal extracts can improve and enhance broiler performance and can be a replacement for antibiotics. The herbal products, ENTERIA, followed by ICEN, improved most of the



studied parameters. Further studies are needed to evaluate the effect of these products on some specific immunological, antioxidant, and microbial parameters of broilers. The use of these products over the entire production period needs to be studied for a better understanding of the effect of these products on general broiler performance.

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#### Authors' contributions

Mustafa and Hassan designed the experiment and collected some of the data; Isa and Mustafa did the histology, serology, and hormonal tests for the collected samples; Hassan performed the data analysis; Isa wrote the draft. All the authors reviewed the final draft.

#### Conflict of interest declaration

The authors declare that there is no conflict of interest regarding this experiment.

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