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AQUEOUS AND ALCOHOLIC PARSLEY (*Petroselinum crispum*) EXTRACTS SUPPLEMENTED INTO DRINKING WATER AND STOCKING DENSITY AFFECT PRODUCTIVE PERFORMANCE AND ANTIOXIDANT STATUS OF BROILERS

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ABSTRACT

Present experiments were conducted to evaluate the effects of aqueous and alcoholic parsley (*Petroselinum crispum*) extracts supplemented into drinking water and stocking density on productive performance (body weight, body weight gain, feed consumption, feed conversion ratio, mortality rate, and production and economic index) and antioxidant status (malondialdehyde, glutathione and catalase) of broilers. Two different stocking densities (10 and 15 chickens / m^2) and 5 different additive levels (0 - control treatment without additives; 4a - addition of 4 milliliters of aqueous extract per liter of water; 4c - addition of 4 milliliters of alcoholic extract per liter of water; 8a - addition of 8 milliliters of aqueous extract per liter of water; 8c - addition of 8 milliliters of alcoholic extract per liter of water) were evaluated. The study included 375 one-day old, unsexed ROSS 308 chicks, and were arranged as 2x5 factorial experiment (viz; factor 1 as two stocking densities and factor two as five additives). The chicks were randomly assigned to ten distinct treatment groups. Each treatment group was further divided into three replicates. Five treatment groups comprised ten chicks each (stocking density 10), while the remaining five groups contained fifteen chicks (stocking density 15). The results showed that treatment 8c was the best regarding weight gain, feed consumption, feed conversion ratio, reduced mortality rates, and production and economic index at both stocking densities. Treatment 4c also showed remarkable effectiveness but was lower than 8c for all studied traits. Based on the overall performance, treatments 4c and 8c are considered the best at a density of 10 chickens/m^2. Present additive treatments reduced blood serum malondialdehyde, glutathione and catalase levels at both stocking densities, indicating a reduction in oxidative stress. In general, the density of 10 chickens was more effective in improving antioxidant indicators as compared to the density of 15 chickens.

Keywords: Aqueous and Alcoholic Extract, Parsley, Stocking Density, Antioxidant Traits, Broiler

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INTRODUCTION

High stocking densities result in serious oxidative stress in poultry farming. Such a stress then results in delayed avian growth, debris and elevated humidity levels in poultry houses. Furthermore, these conditions are the primary cause of respiratory diseases in poultry, such as air vasculitis, when there is a combination of insufficient ventilation and high stocking density. Peck may also occur within the flock due to the high density (Gungor *et al*., 2024).

Antibiotics have traditionally been used in poultry diets to stimulate growth. Nonetheless, the growth of antibiotic-resistant microbes resulted in a restriction on using antibiotic compounds in poultry diets. Furthermore, poultry diets mainly include maize and soybean meals (Jasim and Al-Naif, 2021), which are devoid of antioxidants; consequently, incorporating artificial antioxidants into poultry diets mitigates the adverse effects of rigorous environmental conditions (Traverso *et al.,* 2013). However, research has demonstrated that synthetic antioxidant compounds may have mutagenic or detrimental effects on poultry (Nafea and Ahmed, 2020). As a result, there is an increasing fascination with natural chemicals that promote growth and antioxidants as alternative treatments (Sadeghi *et al*., 2016).

The significance of nutrition research has escalated due to the rising need to boost the productive performance of hens and the prohibition on the use of

antibiotics as growth stimulants (Abo Ghanima *et al*., 2020; Alagawany *et al*., 2021; Elnesr *et al*., 2022). Researchers have been intrigued by naturally occurring bioactive compounds because of the extensively established advantages of dietary supplements that include these organic constituents. The advantages include enhanced growth performance, digestive well-being, immunological reactivity, nutritional assimilation and overall chicken health (Ebrahim *et al*., 2020).

The therapeutic capabilities of medicinal plants are attributed to certain chemical compounds found in plant tissues, which profoundly affect the human body or animals. These substances may include alkaloids, aromatic oils or similar compounds (Sofowora *et al*., 2013). Medicinal plants contain flavonoid compounds, which are highly acknowledged for their value and importance in improving overall health, reducing the risk of diseases and acting as antioxidants that protect against free radicals (Al-Musawi *et al*., 2019).

Parsley (*Petroselinum crispum*) is a prime example of a therapeutic plant or botanical species. The foliage of parsley has more significant amounts of volatile oils than the roots. The leaves contain mineral salts, iron, calcium, phosphorus and vitamins A, C and K. Parsley is thought to possess antioxidant properties due to its many phenolic components and flavonoids. Additionally, it is regarded as advantageous for gastrointestinal well-being and has anti-inflammatory characteristics. Furthermore, it can enhance cardiovascular well-being (Filho *et al*., 2018). The oil derived from the *Petroselinum* plant has antioxidant properties and serves as a suppressor of free radicals; not only does it help combat the harmful effects of free radicals on the body, but it also provides antioxidant benefits (Zhang *et al.*, 2006). Parsley leaves have been discovered to contain an oil called myristicin, with Apiole being the primary constituent in this oil (Razzaghi-Abyaneh *et al.*, 2007). The purpose of this study is to evaluate the effect of aqueous and alcoholic parsley (*Petroselinum crispum*) extracts supplemented into drinking water and stocking density on productive performance and antioxidant status of broilers. This research aims to investigate the bioactive constituents within parsley and their potential mechanisms for modulating growth parameters and enzymatic antioxidant levels. By elucidating these effects, the study seeks to contribute to the development of sustainable, plant-based additives that can enhance poultry health and productivity, potentially reducing the reliance on synthetic growth promoters.

MATERIALS AND METHODS

Ethical approval: All experimental protocols for animal experiments were approved by Protocol No Ref.78 of the Animal Care and Use Committee of Anbar University, Iraq.

Animals and Experimental Design: The study included 375 one-day old unsexed ROSS 308 chicks, randomly distributed in a factorial experiment of two factors (2×5). The first factor is stocking density, which consists of two treatments (10 and 15 birds per square meter). The second factor is additives, which include five treatments (0, 4a, 4c, 8a, 8c), each with three replicates, (0 - control treatment without additives; 4a - addition of 4 milliliters of aqueous extract per liter of water; 4c - addition of 4 milliliters of alcoholic extract per liter of water; 8a - addition of 8 milliliters of aqueous extract per liter of water; 8c - addition of 8 milliliters of alcoholic extract per liter of water). Chicks were randomly assigned to ten distinct treatment groups. Each treatment group was further divided into three replicates, resulting in a total of thirty experimental units. Five treatment groups comprised ten chicks each, while the remaining five groups contained fifteen chicks, thereby ensuring equitable exposure to all treatments. All these additives were supplemented into drinking water of broilers. The chicks were given free access to food and water. Broilers were raised in a temperature-controlled environment and the pen area was 1×1 m. To create a controlled experimental environment, temperature and humidity levels were carefully maintained throughout the 42-day experimental period. The initial temperature was set at 34°C and gradually reduced by 2°C each week until it reached 22°C. Relative humidity was consistently kept within a range of 45 to 55%.

Chicks were meticulously selected based on specific criteria. Only healthy individuals, free from any pre-existing diseases or conditions that could potentially compromise the study's integrity, were included. All chicks were of the same age, one-day at the commencement of the experiment, to establish a uniform baseline for growth and development. Moreover, to eliminate genetic variability as a potential source of variation, chicks were sourced from a single commercial broiler strain, ROSS 308.

Chicks were vaccinated against Newcastle and Gumboro diseases using vaccines diluted in dechlorinated water. The vaccines were administered via either spraying or drinking water. Water was withheld from the chicks for two hours before each vaccination to ensure accurate dosing. Vitamins A, D3, and E were provided after each vaccination.

Growth Performance and Sample Collection: Body weight was recorded weekly from the first week until the sixth week, and weekly weight gain, feed consumption, feed conversion ratio, mortality rate, and relative growth rate were calculated during this period. In addition, cumulative weight gain was calculated over the six weeks. Ultimately, the production index and economic

index were determined based on these data. In the sixth week of the experiment, blood samples were collected to estimate the antioxidant status of blood plasma. About 5.0 ml of blood was randomly drawn from the brachial vein of the wing from one bird per replicate. Blood samples were placed in tubes containing ethylenediaminetetraacetic acid (EDTA). Blood samples were centrifuged at 1500 × g for 10 minutes to separate the plasma. The resulting plasma samples were stored in 1.5 ml microtubes at -20 °C for various antioxidant status tests, including Malondialdehyde (MDA), glutathione, and catalase.

Malondialdehyde (MDA) enzyme was measured according to the method specified in Placer *et al*. (1966). The same method was used to measure the Glutathione enzyme (Fariss and Reed, 1987). The Catalase enzyme was determined according to the method of (Altinordulu and Eraslan 2009).

Preparation of Parsley (*Petroselinum crispum)* extracts: The parsley plant was collected in large quantities and then dried on aluminum foil away from light. There was an airflow, and it was stirred every three hours to speed up the drying process. This process continued until complete drying. After that, dried plants were stored in clean, tightly sealed bags in a clean place at room temperature. Parsley plants were ground using an electric grinder. The extracts were then prepared in two forms: aqueous and alcoholic.

The process of aqueous extraction of parsley: Ten grams of dried parsley powder was immersed into distilled water and cooked over low heat for 2 hours. The mixture was then filtered through eight layers of muslin and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected. The operation was done twice. After 6 hours, the supernatant was collected at 2-hour intervals, pooled, and concentrated to provide a final amount of one-fourth the original volume. It was then autoclaved at 121°C under 15 lb pressure and kept at 4°C (Parekh and Chanda, 2008).

The process of alcoholic extraction of parsley: Ten grams of dried parsley powder were dissolved in 100 ml of organic solvent (ethanol) in a conical flask, plugged with cotton, and shaken at 190-220 rpm for 24 hours. After 24 hours, it was filtered through eight layers of muslin fabric and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected, and the solvent was evaporated to reduce the final volume to one-fourth of the original volume, which was then kept in airtight bottles at 4 °C (Parekh and Chanda, 2008).

Statistical Analysis: Two-way analysis of variance was conducted, first addressing the impact of two stocking density treatments on the measured traits and second examining the influence of four additive factors on the studied traits. This was achieved using the General Linear Model of the statistical software SAS, version 9. Significant differences between means were tested using Duncan's multiple range test (Duncan, 1955) at a significance level of $P \leq 0.05$.

RESULTS

Productive Performance

Body weight (g): Effects of stocking density x additive interactions on body weight of broilers are provided **Table 1**. In the first and third weeks, there were no significant differences between the treatments; however, in the second week, 4c and 8c treatments with density of 10 chickens/m^2 showed significant superiority as compared to the control (0) treatment with density of 10 and 15 chickens/m^2 ($P \leq 0.05$). As for the fourth, fifth and sixth weeks, 4c and 8c treatments with density of 10 and 15 chickens/m^2 showed significant superiority as compared to control treatment (0) with density of 10 and 15 chickens/m^2 ($P \leq 0.05$).

Body weight Gain (g): Effects of stocking density x additive interactions on body weight gain of broilers are given in **Table 2.** There were no significant differences between the treatments in the first, second, third, fifth and sixth weeks. As for the fourth week, additive treatments showed significant superiority as compared to control (0) with density of 10 and 15 chickens/m^2 ($P \leq 0.05$). Except for 4a and 8a treatments with density of 15 chickens/m^2, throughout the entire period, the additive treatments (4a,4c, and 8c) with density of 10 chickens/m^2 and 8c treatment with density of 15 chickens/m^2 showed significant superiority as compared to control treatment with density of 10 and 15 chickens/m^2 ($P \leq 0.05$). Also, 8a treatment with density of 10 chickens/m^2 and 4c treatment with density of 15 chickens/m^2 showed significant superiority as compared to control treatment with density of 10 chickens/m^2 ($P \leq 0.05$).

Feed Consumption (g): Effects of stocking density x additive interactions on feed consumption of broilers are provided in **Table 3.** During the first week, 4c treatment with density of 10 chickens/m^2 was significantly superior as compared to 0, 4a, 4c and 8a treatments with density of 15 chickens/m^2 ($P \leq 0.05$). In the fourth week, 8c treatment with density of 10 chickens/m^2 was significantly superior as compared to all treatments with density of 10 and 15 chickens/m^2 and 4c treatment with density of 10 chickens/m^2 was significantly superior as compared to 8a and 8c treatments with density of 15 chickens/m^2 ($P \leq 0.05$). During the total period, 8c treatment with density of 10 chickens/m^2 was significantly superior ($P \leq 0.05$) as compared to all treatments with density of 15 chickens/m^2. The 4a and 4c treatments with density of 10 chickens/m^2 were significantly superior as compared to all treatments with

density of 15 chickens/m^2 (P≤0.05). There were no significant differences between the treatments during the second, third, fifth and sixth weeks.

Feed Conversion Ratio (g feed / g weight gain): Effects of stocking density x additive interactions on feed conversion ratio of broilers are given in **Table 4.** There was no significant difference between the treatments during the first, second, third and sixth weeks. However, in the fourth week, all the treatments had significant improvement (P≤0.05) as compared to control (0) treatment with density of 10 chickens/m^2. Also, 4c and 8c treatments with density of 10 chickens/m^2 had significant improvement (P≤0.05) as compared to control (0) treatment with density of 15 chickens/m^2. During the fifth week, all treatments showed a significant improvement (P≤0.05) as compared to control treatment (0) with density of 10 chickens/m^2 except for 4a and 8a treatments with density of 15 chickens/m^2. All the treatments significantly improved (P≤0.05) as compared to control treatment (0) with density of 10 chickens/m^2 during the total period.

Relative Growth Rate (%): Effects of stocking density x additive interactions on relative growth rate of broilers are given in **Table 6.** The 4c and 8c treatments with density of 10 chickens/m^2 and 4c with density of 15 chickens/m^2 showed significant superiority as compared to control treatment (0) with density of 10 and 15 chickens/m^2 (P ≤ 0.05). Moreover, 8a treatment with density of 10 chickens/m^2 and 4a and 8c treatments with density of 15 chickens/m^2 showed significant superiority as compared to control treatment (0) with density of 10 chickens/m^2 (P ≤ 0.05). In the total period, 4a, 4c and 8c treatments with density of 10 chickens/m^2 and 4c and 8c treatments with density of 15 chickens/m^2 showed significant superiority as compared to control treatment (0) with density of 10 and 15 chickens/m^2 (P ≤ 0.05). However, there was no significant difference between the treatments through the first, second, third, fifth and sixth weeks.

Production Index: Effects of stocking density x additive interactions on the production index of broilers are given in **Table 7**. The treatments of 4c and 8c with density of 10 chickens/m^2 had a significant superiority (P≤0.05) as compared to all treatments with density of 10 and 15 chickens. Moreover, all treatments, except for 4a with density of 15 chickens/m^2, had a significant superiority (P≤0.05) as compared to control treatment of (0) with density of 10 and 15 chickens/m^2.

Mortality ratio (%): Effects of stocking density x additive interactions on mortality ratio of broilers are provided in **Table 5.** During all the weeks and the total period, there was no significant difference between the treatments.

Table 1. Effects of aqueous and alcoholic extract of parsley (*Petroselinum crispum)* supplemented into drinking water and stocking density on body weight (g) of broilers

	Treatment*		1st week	2nd week	3rd week	4th week	5th week	6th week
	Density	Additives						
Interaction	10	0	189	505^{cd}	1080	1635^{f}	2299^{e}	2937^{e}
		4a	190	513^{cd}	1107	1775^{cde}	2596^{bcd}	3343^{bc}
		4c	199	541^{ab}	1150	1983^{ab}	2776^{ab}	3551^{ab}
		8a	188	526^{abcd}	1122	1812^{cd}	2581^{bcd}	3232^{cd}
		8c	202	549^{a}	1128	2031^{a}	2866^{a}	3694^{a}
	15	0	190	503^{d}	1043	1677^{ef}	2493de	3020de
		4a	198	528^{abcd}	1109	1714^{edf}	2375de	3020de
		4c	192	521^{abcd}	1092	1838^{c}	2546^{cd}	3305^{bcd}
		8a	175	518^{bcd}	1088	1696^{edf}	2376de	3046de
		8c	219	531^{abc}	1147	1874^{bc}	2645^{bc}	3363^{bc}
P-value	Density		NS	NS	NS	0.001	0.004	0.002
	Additives		0.021	0.005	0.030	0001	0.0002	0.0001
	Interaction		NS	0.017	NS	0001	0.0001	0.0001
	SEM		2.93	3.37	8.13	25.2	36.2	49.5

SEM = Standard Error of Means; NS= Non-Significant;
Means in the same column with different superscripts are significantly different (P ≤ 0.05);
*10 or 15 = number of chickens per square meter. 0 = control without additives; 4a or 8a= means 4 or 8 milliliters of aqueous extract per liter of water; 4c or 8c= means 4 or 8 milliliters of alcoholic extract per liter of water.

Table 2. Effects of aqueous and alcoholic extract of parsley (*Petroselinum crispum)* supplemented into drinking water and stocking density on body weight gain (g) of broilers

	Treatment*		1st week	2nd week	3rd Week	4th Week	5th Week	6th week	Total Period
	Density	Additives							
Interaction	10	0	146	316	574	555^{f}	664	638	2895^{e}
		4a	147	322	594	667^{cde}	820	747	3300^{bc}
		4c	156	341	609	832^{ab}	792	774	3508^{ab}
		8a	145	338	596	689^{cde}	769	651	3189^{cd}
		8c	159	346	579	903^{a}	834	828	3651^{a}
	15	0	146	313	540	633^{ef}	816	526	2977de
		4a	155	329	581	604^{ef}	660	651	2984de
		4c	148	329	571	746^{ac}	707	758	3262^{bcd}
		8a	132	343	570	608^{ef}	679	669	3003de
		8c	176	311	615	726^{cd}	771	718	3320^{bc}
P-value	Density		NS	NS	NS	NS	NS	NS	0.002
	Additives		0.025	NS	NS	0001	NS	0.124	0.0001
	Interaction		NS	NS	NS	0001	NS	NS	0.0001
	SEM		2.94	3.75	6.23	20.7	17.2	25.5	49.5

SEM = Standard Error of Means; NS= Non-Significant;
Means in the same column with different superscripts are significantly different ($P \leq 0.05$);
*10 or 15 = number of chickens per square meter. 0 = control without additives; 4a or 8a= means 4 or 8 milliliters of aqueous extract per liter of water; 4c or 8c= means 4 or 8 milliliters of alcoholic extract per liter of water.

Table 3. Effects of aqueous and alcoholic extract of parsley (*Petroselinum crispum)* supplemented into drinking water and stocking density on feed consumption (g) of broilers

	Treatment*		1st week	2nd week	3rd Week	4th Week	5th Week	6th week	Total Period
	Density	Additives							
Interaction	10	0	210^{abc}	509	796	934^{bc}	1209	1068	4729^{abc}
		4a	213^{ab}	493	821	940^{bc}	1198	1297	4953^{ab}
		4c	215^{ab}	511	831	1000^{b}	1213	1193	4965^{ab}
		8a	206^{abc}	501	781	918^{bc}	1218	1032	4662^{bc}
		8c	217^{a}	528	795	1088^{a}	1195	1308	5133^{a}
	15	0	190^{cd}	441	737	886^{c}	1177	941	4375^{c}
		4a	194^{bcd}	489	773	908^{bc}	1144.2	947	4467^{c}
		4c	181^{d}	482	772	914^{bc}	1144.5	1109	4605^{c}
		8a	190^{cd}	494	747	889^{c}	1120	1019	4462^{c}
		8c	214^{ab}	523	762	900^{c}	1101	1129	4633^{bc}
P-value	Density		0.0004	NS	0.001	0.0003	0.018	0.023	0.0004
	Additives		NS	0.171	NS	0.021	NS	NS	0.125
	Interaction		0.008	NS	NS	0.001	NS	NS	0.016
	SEM		2.89	6.65	7.55	13.1	13.4	34.3	57.3

SEM = Standard Error of Means; NS= Non-Significant;
Means in the same column with different superscripts are significantly different ($P \leq 0.05$);
*10 or 15 = number of chickens per square meter. 0 = control without additives; 4a or 8a= means 4 or 8 milliliters of aqueous extract per liter of water; 4c or 8c= means 4 or 8 milliliters of alcoholic extract per liter of water.

Table 4. Effects of aqueous and alcoholic extract of parsley (*Petroselinum crispum)* supplemented into drinking water and stocking density on feed conversion ratio (g feed / g gain) of broilers.

	Treatment*		1st week	2nd week	3rd Week	4th Week	5th Week	6th week	Total Period
	Density	Additives							
Interaction	10	0	1.44	1.61	1.39	1.70^{a}	1.82^{a}	1.70	1.63^{a}
		4a	1.44	1.52	1.38	1.41^{bc}	1.46^{cd}	1.71	1.50^{b}
		4c	1.37	1.49	1.36	1.20^{d}	1.53^{bcd}	1.54	1.41^{b}
		8a	1.42	1.48	1.31	1.33^{cd}	1.59^{bcd}	1.67	1.46^{b}
		8c	1.36	1.52	1.37	1.20^{d}	1.43^{d}	1.60	1.40^{b}
	15	0	1.29	1.40	1.36	1.39^{bc}	1.47^{cd}	1.78	1.47^{b}
		4a	1.25	1.48	1.33	1.50^{b}	1.73^{ab}	1.56	1.50^{b}
		4c	1.22	1.46	1.35	1.22d	1.61^{bcd}	1.46	1.41^{b}
		8a	1.50	1.45	1.31	1.46^{bc}	1.65^{abc}	1.52	1.48^{b}
		8c	1.22	1.69	1.24	1.24^{d}	1.42^{d}	1.58	1.39^{b}
P-value	Density		0.028	NS	NS	NS	NS	NS	NS
	Additives		NS	NS	NS	0.001	NS	NS	0.001
	Interaction		NS	NS	NS	0001	0.002	NS	0.003
	SEM		0.026	0.025	0.013	0.031	0.028	0.045	0.015

SEM = Standard Error of Means; NS= Non-Significant;
Means in the same column with different superscripts are significantly different ($P \leq 0.05$);
*10 or 15 = number of chickens per square meter. 0 = control without additives; 4a or 8a= means 4 or 8 milliliters of aqueous extract per liter of water; 4c or 8c= means 4 or 8 milliliters of alcoholic extract per liter of water.

Table 5. Effects of aqueous and alcoholic extract of parsley (*Petroselinum crispum)* supplemented into drinking water and stocking density on mortality ratio (%) of broilers.

	Treatment*		1st week	2nd week	3rd Week	4th Week	5th Week	6th week	Total Period
	Density	Additives							
Interaction	10	0	0	0	0	0	0	0	0
		4a	0	0	0	0	3.33	0	3.33
		4c	0	0	0	0	0	0	0
		8a	0	0	0	0	0	3.33	3.33
		8c	0	0	0	0	0	0	0
	15	0	0	0	0	0	6.66	0	6.66
		4a	0	0	0	0	0	4.44	4.44
		4c	0	0	0	0	0	0	0
		8a	0	0	0	0	0	0	0
		8c	0	0	0	0	0	0	0
P-value	Density		NS	NS	NS	NS	NS	NS	NS
	Additives		NS	NS	NS	NS	NS	NS	NS
	Interaction		NS	NS	NS	NS	NS	NS	NS
	SEM		0	0	0	0	0.735	0.546	0.886

SEM = Standard Error of Means; NS= Non-Significant;
*10 or 15 = number of chickens per square meter. 0 = control without additives; 4a or 8a= means 4 or 8 milliliters of aqueous extract per liter of water; 4c or 8c= means 4 or 8 milliliters of alcoholic extract per liter of water.

Table 6. Effects of aqueous and alcoholic extract of parsley (*Petroselinum crispum)* supplemented into drinking water and stocking density on relative growth rate (%) of broilers.

	Treatment*		1st week	2nd week	3rd week	4th Week	5th Week	6th week	Total Period
	Density	Additives							
Interaction	10	0	126	91.05	72.3	40.7^{e}	33.8	24.46	194.26^{e}
		4a	126	91.6	73.2	46.2^{cde}	37.5	25.04	194.91^{abc}
		4c	129	92.2	72.02	53.1^{ab}	33.3	24.45	195.22^{ab}
		8a	125	94.6	72.2	46.9^{cd}	34.9	22.46	194.75^{cde}
		8c	129	92.1	69.01	57.1^{a}	34.0	25.25	195.36^{a}
	15	0	125	90.3	69.8	46.5^{cde}	38.8	19.09	194.34^{de}
		4a	128	90.7	71.03	42.8^{de}	32.3	23.90	194.39^{de}
		4c	126	92.3	70.7	50.8^{bc}	32.2	25.93	194.83^{cd}
		8a	120	99.3	70.9	43.6^{de}	33.3	24.72	194.46^{cde}
		8c	134	83.1	73.3	48.1^{bcd}	34.1	23.89	194.96^{ab}
P-value	Density		NS	NS	NS	0.044	NS	NS	0.004
	Additives		NS	NS	NS	0.0001	NS	NS	0.0001
	Interaction		NS	NS	NS	0.0001	NS	NS	0.0003
	SEM		1.02	1.17	0.465	0.994	0.593	0.762	0.077

SEM = Standard Error of Means; NS= Non-Significant;
Means in the same column with different superscripts are significantly different ($P \leq 0.05$);
*10 or 15 = number of chickens per square meter. 0 = control without additives; 4a or 8a= means 4 or 8 milliliters of aqueous extract per liter of water; 4c or 8c= means 4 or 8 milliliters of alcoholic extract per liter of water.

Table 7. Effects of aqueous and alcoholic extract of parsley (*Petroselinum crispum)* supplemented into drinking water and stocking density on production index and economic index of broilers.

	Treatment*		Production Index	Economic Index
	Density	Additives		
Interaction	10	0	428^{f}	428^{e}
		4a	511^{d}	530^{bcd}
		4c	598^{ab}	598^{ab}
		8a	508^{d}	526^{bcd}
		8c	626^{a}	626^{a}
	15	0	452^{f}	490^{cde}
		4a	458^{ef}	483de
		4c	557^{c}	557^{abc}
		8a	488de	488^{cde}
		8c	574^{bc}	574^{ab}
P-value	Density		0.0009	NS
	Additives		0.0001	0.0001
	Interaction		0.0001	0.0001
	SEM		12.09	12.06

SEM = Standard Error of Means; NS= Non-Significant;
Means in the same column with different superscripts are significantly different ($P \leq 0.05$);
*10 or 15 = number of chickens per square meter. 0 = control without additives; 4a or 8a= means 4 or 8 milliliters of aqueous extract per liter of water; 4c or 8c= means 4 or 8 milliliters of alcoholic extract per liter of water.

Economic Index: Effects of stocking density x additive interactions on economic index of broilers are provided in Table 7. The treatments of 4c and 8c with density of 10 chickens/m^2 and 8c with density of 15 chickens/m^2 had a

significant superiority (P≤0.05) as compared to control treatment with density of 10 and 15 chickens/m^2. Moreover, 4a and 8a treatments with density of 10 chickens/m^2 and 4c with density of 15 chickens/m^2 had a significant superiority (P≤0.05) as compared to control treatment (0) with density of 10 chickens/m^2.

Antioxidant Status in Blood Plasma: Effects of stocking density and additive interactions on blood plasma antioxidant status of the broilers are given in **Table 8**. In terms of malondialdehyde and catalase levels, all treatments with density of 10 and 15 chickens/m^2 showed a significant decrease (P≤0.05) as compared to control (0) and 8a treatments with density of 15 chickens/m^2. As for the glutathione levels, all additive treatments with density of 10 and 15 chickens/m^2 showed a significant decrease (P≤0.05) as compared to control treatment (0) with density of 15 chickens/m^2, but 8a with density of 15 chickens/m^2 was not significantly different from control treatment (0) with density of 15 chickens/m^2.

Table 8. Effects of aqueous and alcoholic extract of parsley (*Petroselinum crispum*) supplemented into drinking water and stocking density on blood plasma antioxidant status of broilers.

	Treatment*		Malondialdehyde (nmol/mL)	Glutathione (U/mL)	Catalase (U/mL)
	Density	Additives			
Interaction	10	0	0.440^{b}	35.4^{c}	40.7^{b}
		4a	0.446^{b}	53.3^{bc}	18.1^{b}
		4c	0.430^{b}	31.4^{c}	20.6^{b}
		8a	0.410^{b}	34.1^{c}	21.3^{b}
		8c	0.486^{b}	36.9^{c}	19.7^{b}
	15	0	0.770^{a}	78^{a}	84.6^{a}
		4a	0.523^{b}	52.4^{bc}	37.7^{b}
		4c	0.566^{b}	34.1^{c}	16.9^{b}
		8a	.0450^{a}	72.4^{ab}	76.5^{a}
		8c	0.426^{b}	34.1^{c}	19.3^{b}
P-value	Density		0.002	0.001	0.0001
	Additives		0.016	0.004	0.0001
	Interaction		0.001	0.0003	0.0001
	SEM		0.022	3.52	4.83

SEM = Standard Error of Means; NS= Non-Significant;
Means in the same column with different superscripts are significantly different (P ≤ 0.05);
*10 or 15 = number of chickens per square meter. 0 = control without additives; 4a or 8a= means 4 or 8 milliliters of aqueous extract per liter of water; 4c or 8c= means 4 or 8 milliliters of alcoholic extract per liter of water.

DISCUSSION

Productive Performance: Medicinal plants possess therapeutic capabilities because of certain chemical compounds in their tissues that might affect the human body or animals. These chemicals include alkaloids, flavonoids, essential oils, and other bioactive compounds (Vaou *et al*., 2022). Medicinal plants contain flavonoids, potent chemicals known for enhancing health, reducing illness risk, and acting as antioxidants to combat free radicals (Akbari *et al.*, 2022). One of these herbs and medicinal plants is the herb parsley, also known as *Petroselinum*, which has leaves that contain more volatile oils than its roots. The leaves contain mineral salts, iron, calcium, phosphorus, and vitamins A and C (Al-Musawi *et al*., 2019). Parsley's potent chemicals that enhance digestion resulted in significant superiority in body weight and weight gain (Santos *et al*., 2021). Moreover, these chemicals function as growth stimulants and enhance the activity of digestive enzymes (Al-Musawi *et al*., 2019). Therefore, the nutritious content of feed items should be maximized.

Parsley may contribute to increased live weight by regulating digestion and metabolism, as its leaves contain flavonoids that serve as natural antioxidants. It activates enzymes like Glutathione Peroxidase, which safeguards tissues against peroxides and inhibits the breakdown of body proteins, thus promoting body weight and weight gain in birds (Dorman and Deans, 2000). Also, dried parsley leaves contain volatile oil with active chemicals Myristicin and Apiole, which are the plant's active components (Razzaghi-Abyaneh *et al*., 2007). This

process enhances protein and fat digestion in birds by promoting the release of digestive juices in the digestive tract, maximizing the utilization of essential nutrients for vital functions and chemical reactions (Bahnas *et al*., 2009). The potential enhancement in body weight and weight gain for the treatment of aqueous and alcoholic extracts may be attributed to active compounds, specifically phenolic compounds, which possess antioxidant properties akin to those of vitamin E. These compounds promote intestinal flora within the digestive tract, stimulating the activity of digestive enzymes such as Amylase, Trypsin, Chymotrypsin, and Lipase. Consequently, this enhances the efficiency of nutrient digestion and absorption, increasing body weight and weight gain (Wang *et al*., 2022). Flavonoids also exhibit a structure and function similar to steroid hormones (Harborne, 1975). Steroid hormones boost nutritional metabolism by acting as structural hormones that enhance body growth, stimulate the production of structural proteins in muscles, and decrease protein breakdown (Islam *et al*., 2022).

As for feed consumption, the augmentation in feed consumption for Parsley's aqueous and alcoholic extract may be attributed to the elevated levels of active chemicals and flavonoids in the extract. These compounds exhibit antibacterial properties and can disrupt pathogenic fungi's cellular membranes, exhibiting antioxidant activity (Zhang *et al*., 2006). Parsley is abundant in apigenin, a potent antibacterial agent that effectively combats germs, including *Escherichia coli*, *Salmonella typhi*, and *Candida albicans*. This enhances digestive efficiency and promotes feed consumption by reducing the presence of dangerous microbes (Al-Musawi *et al*., 2019). Parsley oil has properties that strengthen the immune system because it contains volatile oils such as α-Pinene, a Piol, and Myristicin, which has shown effectiveness in transporting an enzyme glutathione-s-transferase which helps bind glutathione with oxidative molecules that damage the cellular membrane (Mahmood *et al*., 2013). Since parsley oil contains the compound limonene, which is rich in vitamin C, it is beneficial for birds' health, as it reduces stress and thus increases feed consumption. It also contains β-carotene, which is converted into Vitamin A, as it maintains the cell membrane of all tissues (Mahmood *et al*., 2013).

As for the feed conversion ratio, the observed enhancement in the feed conversion ratio in both the aqueous and alcoholic parsley extracts may be related to active compounds that enhance the resilience of gut tissue. This, in turn, results in improved utilization of feed components and increased metabolic rates (Škerget *et al.*, 2005). Additionally, there is an enhancement in the efficiency of digestive enzyme release in the digestive system and the maintenance of microbial equilibrium in the gut (Gülçin *et al*., 2004). The observed reduction in mortality rates and improvement in relative growth may be attributed to active constituents in parsley. These constituents possess antimicrobial and antioxidant properties, which contribute to improving intestinal health and mitigating disease risk in broilers. Consequently, this improvement in intestinal health facilitates enhanced nutrient absorption, promoting improved growth and overall health outcomes (Al-Musawi *et al*., 2019). Medicinal plants contain flavonoids, potent chemicals known for enhancing health, reducing illness risk, and acting as antioxidants to combat free radicals; thus, they reflect positively on public health (Akbari *et al*., 2022).

The parsley plant includes unsaturated fatty acids like palmitic and oleic acids, along with phenolic substances such as carvacrol and geraniol, which contribute to its antioxidant properties, ability to combat free radicals, and function as an antidote against dangerous germs and fungus. Thus, parsley extracts improved production index and economic index values. Parsley contains flavonoids and secondary metabolites that enhance digestion and absorption by stimulating the secretion of pancreatic enzymes such as amylase and protease, this process improves protein digestion, converting them into amino acids and increasing their absorption efficiency from the digestive tract, ultimately promoting body weight and weight gain, enhancing the efficiency of feed conversion and thus reflecting positively on the productive and economic indicator (Abbas, 2010). Superiority of the aqueous and alcoholic extract of parsley is attributed to the ability of active compounds, nutrients and vitamins of parsley. In addition, bioactive compounds of parsley, such as citral, can hinder the growth of microorganisms. All of these worked to enhance the general health of broilers, improve digestibility, and increase the benefit of nutritional elements. This led to an increase in productive performance indicators such as live body weight, weight gain and feed conversion, which reflected positively on the production index and the economic indicator (Mahmood *et al.*, 2013). Research has proven that including parsley in bird diets enhanced the productive performance of broilers (Abbas, 2010).

Blood Plasma Antioxidant Status of Broilers: Because medicinal plants contain certain chemical compounds in their tissues, they have the potential to be therapeutically effective. These chemicals may affect the human body or animals. For example, alkaloids, flavonoids, essential oils, and other bioactive compounds are included in this category of chemicals (Vaou *et al*., 2022).

Flavonoids are potent molecules found in medicinal plants. They are renowned for improving health, lowering the risk of sickness, and serving as antioxidants to battle free radicals (Akbari *et al*., 2022).

Some therapeutic plants and herbs include the herb parsley, also called *Petroselinum*. The leaves of this plant contain more volatile oils than the roots. In addition

to vitamins A and C, the leaves provide a source of mineral salts, iron, calcium, and phosphorus (Al-Musawi *et al*., 2019). The decrease in malondialdehyde may be due to the treatments in which the aqueous and alcoholic extract of parsley was used because parsley is an antioxidant, and parsley leaves contain various active substances that, in turn, act as antioxidants. It is rich in vitamin C and phenolic substances (phenols and flavonoids) (Al-Musawi *et al*., 2019). Antioxidants work to restrict free radicals within the body, including their containment of hydroxyl groups (Landete, 2012). Antioxidants inhibit the activity of enzymes that encourage oxidation, including (NAD(P) H oxidase, xanthine oxidase, and protein kinase), which participate in the electron transport chain within the cell, thus preventing fat oxidation from occurring (Procházková, 2010). As for the glutathione and catalase levels in blood serum, the decrease in glutathione levels may be due to it being the first line of defense against oxidative stress (Ram Shetty *et al.*, 2013). Glutathione is one of the most important antioxidant compounds that protect cells from free radicals. Its decrease in blood serum may be due to its association with fat oxidation products (Traverso *et al*., 2013). This is, glutathione (GSH) is often converted by cells in hydrolysis into amino acids or individual peptides and then contributes to the construction of proteins, and this, in turn, is reflected in the body weight and thus an increase in body weight. As for the nonsignificant increase in antioxidant enzymes, research has shown that the effectiveness of antioxidant enzymes varied from one strain to another (Farahat *et al.,* 2008).

Conclusions: It was concluded based on present findings that stocking density had an essential effect on the overall performance of broilers. Treatments involving 4 and 8 milliliters of alcoholic extract per liter of water yielded better outcomes at a stocking density of 10 chickens/m^2 as compared to 15 chickens/m^2. Stocking density of 10 chickens/m^2 contributed to improving productive performance indicators in addition to enhancing the antioxidant status in blood plasma and reducing oxidative stress. It is recommended to adopt a relatively low stocking density (10 chickens per m^2) to maximize the overall performance of broilers by using 8 millilitres of alcoholic extract per liter of water.

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