

Effect of dietary supplementation of wild leek (*Allium tricoccum*) and garlic (*Allium sativum*) leaves on production, egg quality, serum lipid profile, intestinal morphology and digestibility of laying quails

Zana Azeez Abdulkareem

zana_med@uor.edu.krd

University of Raparin

Research Article

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Abstract

In this study, the effects of adding dried wild leek and garlic leaves to the diet of laying quails on egg quality, production performance, intestinal histomorphology, digestibility, and serum biochemical traits were evaluated. 168 – 30 weeks aged- laying quails were randomly arranged into 7 treatments (control group and three different levels (0.5%, 1%, and 1.5%) of wild leek or garlic leaves), 6 replicates, and 4 quails per pen. According to the study, the highest hen-day production was achieved by adding 0.5% garlic leaves, and the egg weight was higher with 1% garlic leaves and 0.5% wild leek compared to the control group ($P < 0.05$). In addition, adding garlic leaves decreased the average daily feed intake and increased high-density lipoprotein concentration. However, adding both herbs did not influence yolk height, albumin height, shell thickness, and Haugh unit ($P > 0.05$). All supplemented treatments significantly increased the Rouch index compared to the control. Quails fed with 1% garlic leaves experienced serum cholesterol, very low-density lipoprotein, low-density lipoprotein, and uric acid. Moreover, three levels of garlic leaves decreased Triglyceride concentration. Adding 0.5% and 1% garlic leaves to the diet could increase the digestibility of ash, crude protein, jejunal, and ileal villus height-to-crypt depth ratio. Furthermore, 1% and 1.5% garlic leaves supplementation elevated organic matter digestibility. In conclusion, supplementing 1% garlic leaves to the quail's diet could improve egg weight, intestinal morphology, and nutrient digestibility, improving lipid profiles and reducing uric acid concentration in the serum. However, both plants were rich pigment sources, increasing the rouch index.

1. Introduction

Poultry industries rapidly expand to provide a growing global protein source for an ever-increasing world population. While chicken production and products remain the most common, in recent years, Japanese quails have emerged as an alternative source of high-quality meat and eggs. Japanese quails have several advantages, including a rapid growth, sexual maturity at a young age, short generation interval with a brief incubation period, and a high egg production rate (Panda and Singh, 1990; Reddish et al., 2003). According to Sezer (2007), quail hens can lay between 280–300 eggs in their first year under favourable conditions and environments.

On the other hand, poultry production faces several challenges, including poor nutrition, inadequate husbandry practices, lack of proper housing, and sanitation issues, as highlighted by Alemneh and Getabalew (2019), and Mengesha et al. (2022). These challenges lead to a high disease burden and the emergence of new pathogens, which in turn encourage farmers to use antibiotics to prevent and treat diseases (Afakye et al., 2020; Paintsil et al., 2021). Low doses of antibiotics used as growth promoters have led to bacterial resistance (WHO, 2017). Bacterial resistance is a serious problem, as reported by O'Neill (2016), who predicted that bacterial antibiotic resistance could cause the death of nearly 10 million people in the United Kingdom by 2050. To reduce the risk of antibiotic resistance, natural additives such as medicinal herbs are considered a viable alternative to antibiotics (Joint, 2019).

The *Allium* genus, which belongs to the Amaryllidaceae family, has a global presence and has been used for various therapeutic purposes and is used as feed and spices by humans, as per studies conducted by Bongiorno et al. (2008), Ali and Zahran (2010), Ogbuewu et al. (2019), Ogbuewu et al. (2021), and Malematja et al. (2022). Garlic (*Allium sativum*) is the oldest plant species known to have been cultivated by humans for therapeutic purposes. Its essential oils contain allicin ($C_6H_{10}S_2O$) as the principal compound, which has several biological properties, such as antibiotics, antifungal, and antiviral (Leontiev et al., 2018).

Due to its medicinal properties, garlic can be an alternative to medicine (Bongiorno et al., 2008; Ogbuewu et al., 2019). Garlic has been included as a supplement in broiler chicken diets for at least three decades (Leontiev et al., 2018). Kairalla et al. (2022) studied the impacts of adding garlic powder to the diets of broiler chickens. They showed improved growth performance, serum lipid profile, hematocrit, and total white blood cell count. As previously researched, garlic contains organosulfur compounds, including Allicin, diallyl sulfide, diallyl trisulphide, and allyl mercaptan. These bioactive substances make garlic more effective in affecting almost all body systems (Kothari et al., 2019; Ogbuewu et al., 2019; Puvača et al., 2015).

Garlic has gained significant attention in the poultry egg production industry, particularly concerning laying hens. A study conducted by Omer et al. (2019) investigated the effect of garlic on laying hens and found that garlic powder can enhance egg production and weight, improve the feed conversion ratio, promote better health in hens, and reduce cholesterol levels. Another meta-analysis by Ogbuewu et al. (2021) reported that garlic supplementation in laying chickens can increase daily egg production, egg weight, eggshell weight, and thickness. Further studies are essential to understand garlic's effects on laying quails adequately. Several research studies have suggested that the inclusion of garlic powder in the diet of quail hens leads to a reduction in both serum and egg yolk cholesterol levels. Notably, this supplementation does not exhibit any adverse effects on their overall performance or the quality of

eggs produced (Canogullari et al., 2010; Yalcin et al., 2007). Additionally, it has been observed that garlic powder supplementation can improve the productive and reproductive traits of quails (Ismael and Ameen, 2022).

Garlic leaves are one of the side wastes of garlic that are abundantly separated from the tuber during harvest and are usually used as compost or burned, and this can increase the cost of production waste disposal and lead to environmental pollution. Using garlic leaves as an additive in poultry feed is an effective and promising way to use waste for biological production (Kamruzzaman et al., 2014). Fukaya et al. (2020) investigated that the effective constituents of garlic leaves are similar to the tuber. As shown in this study, five cyclic organosulfurs were identified in garlic leaf extract, all di or trisulfides. So, it is expected to have the same positive effects as the tuber. For the above reasons, we use garlic leaves instead of garlic tuber in the current study.

Wild leeks (*Allium tricoccum*), a perennial herb with garlic and onion flavour, are grown in moist, shady mountains and belong to the *Allium* genus. Traditional folk medicine suggests wild leek to lower blood pressure, lipids, and cholesterol while increasing blood circulation (Cavender, 2006). Dabeek et al. (2019) discovered that wild leek contains flavonoids, specifically kaempferol and quercetin, mainly present in the leaves and not found in the bulb. The conjugates of these flavonoids are also present, but they are more evenly distributed in the leaves than in the stem. Poultry growth performance, blood constituents, intestinal health, and carcass composition improve with increased flavonoid intake, as their antioxidant, antimicrobial, and antiviral properties are beneficial (Prihambodo et al., 2021).

Studies have shown that garlic and onion bulbs benefit broilers and laying chickens. Nevertheless, there exists a scarcity of research investigating the impact of garlic leaves specifically on the laying quails. Garlic leaves and wild leek belong to the Alliaceae family and may improve the health and performance of laying quails, but they have not been studied before. Hence, this study aimed to assess the influence of garlic and wild leek leaves on egg production and quality, performance, blood serum lipid profile, intestinal morphology, and food digestibility in Japanese quails.

2. Materials and methods

2.1. Plant additive preparation and analysis

Local farmers supplied wild leek and garlic leaves, which were processed into a fine powder using a laboratory mill. The powdered form underwent analysis for crude protein, ether extract, crude fiber, and ash content through laboratory procedures following AOAC guidelines (Horwitz, 2010).

Birds, housing and feeding

The study took place at the Animal Science Department of the University of Raparin in Ranya, Sulaymanayah, Iraq. All protocols for animal care and usage underwent assessment and approval by the University of Raparin Animal Care and Ethical Committee, adhering to global standards (FASS., 2012). The study used 168 laying quails (*Coturnix coturnix*) aged 30 weeks, which had been in production for 24 weeks. At the onset of the trial, the birds were collectively weighed and then allocated into 42 cages outfitted with a gutter feeder and nipple drinking system. The 7 different treatments were organized following a Completely Randomized Design for a duration of 10 weeks. Each treatment consisted of 6 replicates, each housing 4 quails. Both feed and water were provided unrestrictedly (ad libitum), with the feed being given in a mashed form.

Table 1 displays the fundamental experimental diets created to fulfill the nutritional needs of laying quail as per the specified requirements according to Council (1994) The experimental diets consisted of control and three inclusion levels (0.5%, 1%, and 1.5%) of wild leek or garlic leaves. The nutrient composition and ingredients of the diets are provided in Table 1. The lighting schedule followed a 16-hour light and 8-hour dark cycle, while keeping the temperature within the range of 22-26°C and maintaining the relative humidity between 65-75%.

Table 1 is here

2.2. Performance measurements

The experiment started at 30 weeks old, with body weight recorded. Over the following 10 weeks, egg production, feed consumption, egg weight, and mortality were closely monitored and recorded. The experimental diets were evaluated based on the average weight

of the eggs (grams), hen day production (%), egg mass (grams/quail/day), average daily feed intake (ADFI) (grams), and feed conversion ratio (FCR) (calculated as the average feed intake divided by egg mass).

2.3. Egg quality

Egg quality assessments were carried out on pairs of eggs collected on two consecutive days, done biweekly at the ages of 32, 34, 36, 38, and 40 weeks of the hens. The eggs were measured for length and width to determine the egg shape index. After separating yolks and albumins, the height of each was measured to determine their relative weight, yolk-to-albumin ratio, Haugh unit, the relative weight of the dry shell, and the rough index.

2.4. Blood and serum collection

At 40 weeks of age, blood samples were gathered from the brachial vein of one quail per replication. Post-collection, the samples underwent centrifugation at 4000 rpm for 10 minutes to isolate the serum. The sera were kept at a temperature of -20° C until needed for analysis. The levels of serum cholesterol, triglycerides, high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and uric acid were then measured using diagnostic colourimetric kits from Biolabo S.A.S company, France.

2.5. Carcass traits

At the end of the tenth week (at 40 weeks of age), the hens allocated for blood sampling were measured and humanely euthanized using cervical dislocation. Subsequently, the weight of the carcass, liver, gizzard, pancreas, and heart were quantified. Additionally, the weight of various portions of the intestine, specifically the duodenum, jejunum, ileum, and ceca, were documented, and all weight measurements were calculated as a percentage of the live body weight.

2.6. Intestinal morphology

A segment approximately 2 cm away from the midpoint of both the jejunum and ileum underwent rinsing with a normal saline solution before being preserved in 10% formalin. The tissues were treated using an automated tissue processor and then enclosed in paraffin wax for preservation. Sections were then cut to a thickness of 6µm using a microtome (Model Histo-Line, Italy). Subsequent to slide mounting, the specimens were subjected to hematoxylin and eosin staining, followed by the determination of morphometric parameters under a light microscope at a 40X magnification. These indices included villus height, crypt depth, villus height to crypt depth ratio, and villus surface area. The average of 10 villi per slide was used for further analysis (Abdollahi et al., 2021).

2.7. Apparent ileal digestibility

As an indigestible marker, Merck Company's titanium dioxide (TiO₂) was added to the diets at a rate of 3 g/kg four days before the end of the test. After slaughtering the quails, their total ileum contents were collected in labelled plastic bags and immediately stored at -20°C until analysis. The samples' dry matter, organic matter, ash, and crude protein were determined using the Horwitz (2010), and the apparent ileal digestibility was calculated using the Short et al. (1996) method.

2.8. Statistical analysis

The gathered data underwent analysis of variance employing the General Linear Model procedures of SAS (2023). as a Completely Randomized Design. Tukey's test was utilized to discern disparities between means upon significance declaration of the model.

3. Results

3.1. Plant additive Preparation and Analysis

Table 2 is here

Table 2 displays the chemical makeup of the wild leek and garlic leaf samples utilized in this investigation, showcasing their crude protein, crude fiber, ether extract, nitrogen-free extract, and ash content on a dry matter basis.

3.2. Ethanolic extract of garlic and wild leek leaves

The ethanolic extract of garlic leaves primarily contains isopentyl formate, the most abundant active compound at 86.451%. The remaining 13.549% of bioactive compounds are sulfide agents, including Diallyl trisulfide, Allyl methyl trisulfide, and Diallyl disulfide, as shown in Table 3 and Figure 1.

The active compounds and GC-MS graphical picks of wild leek ethanolic extract are presented in Table 4 and Figure 2. The most abundant active compounds found in wild leek are Dipalmitin, Chlorozotocin, Muramic acid, Tetracontane-1,40-diol, Butyl 9,10-epoxystearate, and 2-Myristynoyl pantetheine, with percentages of 37.300%, 17.928%, 9.843%, 7.589%, and 5.283%, respectively.

Tables 3 and 4 are here

Figures 1 and 2 are here

3.3. Performance

Table 5 exhibits the impact of the dietary treatments on hen-day production, egg weight, egg mass, ADFI, and FCR. The highest and lowest hen-day productions were recorded at the supplementation of 0.5% and 1.5% garlic leaves, respectively ($P < 0.05$). However, there were no notable distinctions observed between the remaining treatments and the control group. Quails fed with diets containing 1% garlic leaves and 0.5% wild leek had heavier egg weights than the control, while those fed with 1.5% wild leek had the lightest egg weight among treatments ($P < 0.05$). Adding 1.5% wild leek to the quail diet significantly decreased egg mass compared to the 0.5% garlic leaves treatment. ADFI decreased in quails fed with 1.5% garlic leaves ($P < 0.05$). No statistically significant differences were noted among the treatments concerning FCR ($P > 0.05$).

Table 5 is here

3.4. Egg quality

Table 6 is here

Table 6 demonstrates the influence of dietary treatment on egg quality. Findings indicate that the dietary treatments did not produce a significant impact on egg shape index, yolk weight, albumin weight, yolk-to-albumin ratio, and shell weight ($P > 0.05$). Also, the yolk height, albumin height, shell thickness, and Haugh unit were not changed by adding garlic leaves and wild leek to the diet ($P > 0.05$). However, adding 1% garlic leaves to the diet reduced ($P < 0.05$). The supplementation in all treatments led to a notable rise in the Rouch index compared to the control group, displaying statistical significance ($P < 0.05$).

3.5. Serum biochemical traits

The effects of different dietary treatments on serum cholesterol, triglycerides, HDL, VLDL, LDL, and uric acid levels are presented in Table 7. The findings indicated that quails given 1% garlic leaves experienced a significant reduction in serum cholesterol, VLDL, and uric acid levels in comparison to the control group ($P < 0.05$). Furthermore, LDL levels decreased significantly with the addition of 1% garlic and wild leek leaves compared to the control group ($P < 0.05$). Triglyceride levels exhibited a notable reduction in all three groups that received supplementation with garlic leaves, displaying statistical significance ($P < 0.05$). In addition, the HDL

Table 7 is here

concentration was significantly increased when the diet was supplemented with 1.5% garlic ($P < 0.05$).

3.6. Carcass traits

Table 8 is here

Table 8 delineates the impact of dietary treatments on the proportional weight of carcasses and gastrointestinal components concerning live body weight. The supplementation of dietary treatments did not produce any significant effects on the proportional weights of the carcass, liver, gizzard, pancreas, heart, duodenum, jejunum, ileum, or cecum ($P > 0.05$).

3.7. Intestinal morphology

Table 9 is here

The effect of experimental treatments on jejunal and ileal villus height, crypt depth, villus height-to-crypt depth ratio, and villus surface area are shown in Table 9. Adding 0.5% garlic leaves could increase the jejunal villus height-to-crypt depth ratio ($P < 0.05$). Also, the ileal villus height-to-crypt depth ratio increased due to adding 0.5 and 1% garlic leaves to the quail diet ($P < 0.05$). Dietary treatments did not affect jejunal and ileal villus height, crypt depth, and villus surface area ($P > 0.05$).

3.8. Apparent ileal digestibility

The impact of treatments on the digestibility of ileal dry matter, organic matter, protein, and ash is outlined in Table 10. As shown, treatments did not influence dry matter digestibility, but supplementation of 1 and 1.5% garlic leaves elevated OM digestibility ($P < 0.05$). In addition, levels of 0.5 and 1% garlic leaves could increase ash and crude protein digestibility ($P < 0.05$).

Table 10 is here

4. Discussion

According to the findings of this study, incorporating 0.5% garlic leaves into the quails' diet resulted in a numerical rise in both egg production and egg mass compared to both the control group and other treatments. Studies conducted by previous researchers have had various results. For example, Canogullari et al. (2010) and Khan et al. (2007) conclusively demonstrated that supplementing laying hens' diet with garlic powder significantly increased egg production. Olayinka et al. (2022a) reported that adding 0.75% garlic powder to quail's diet significantly increased hen-day egg production. However, Chowdhury et al. (2002) have conclusively demonstrated that supplementing chicken feed with garlic powder does not impact egg production. This difference in the results can be due to the diversity in the effective metabolites content of the plant used, level used, bird kind or age.

Increasing egg weight after supplementing 1% garlic leaves and 0.5% wild leek is due to their antimicrobial components. These findings align with Yalcin et al. (2006), who showed that adding 5 and 10 g of garlic to a kilogram of laying hen diets increased egg weight. Allium families, such as garlic and wild leek, contain sulfur compounds and volatile components such as thiosulfate and allicin (Calvey et al., 1998) that, with their antimicrobial effect, improve the health of the digestive system and subsequently increase the performance of the bird. Their discovery revealed that the primary sulfur compound in ramp bulbs obtained from West Virginia, at a rate of 3 $\mu\text{mol/g}$ dry weight, was the thiosulfinate known as allicin. Aswal et al. (2017) found that allicin enhanced egg production. Zennie and Ogzewalla (1977) reported that Wild leek leaves gathered from southern Ohio and northern Kentucky possess 0.8 mg/g of vitamin C in dry weight, exhibiting both antimicrobial and antioxidant properties. Adding garlic leaves at a high dose (1.5%) reduces daily feed intake and egg production in laying quails compared to a lower amount (0.5%) significantly and numerically compared to the control group, which also reduced daily feed intake and egg production level. The results partially agree with Lee et al. (2016), which reported that adding garlic to the diet did not influence feed intake and performance. However, Abdulkareem et al. (2023) reported the adverse effect of Allium on broiler performance, and Canogullari et al. (2010) and Olobatoke and Mulugeta (2011) demonstrated that garlic powder reduces egg production in quail and chicken by 4 to 5%. The reduction in feed consumption and egg production may return to the presence of bioactive compounds in the garlic, particularly Diallyl trisulfide, Allyl methyl trisulfide, and Diallyl disulfide. Because several studies demonstrate the negative impact of sulfide agents on feed intake, weight gain, and egg production, such as Wang et al. (2011) reported that sulfides significantly decline daily feed intake and body weight in broiler chicken at 8 g/kg level. Also, Kim et al. (2021) evaluated the effect of sulfide agents on laying chickens and reported that sulfide agents decreased feed intake and egg production. Chan et al. (2007) and Slade et al. (2018) also stated that diallyl trisulfide-rich garlic oil and sulfides suppress feed consumption by inhibiting ghrelin hormone secretion, reducing feed intake and body weight.

There was a significant increase in egg weight after adding garlic leaves and wild leek in small quantities. This finding aligns with Mahmoud et al. (2006) research and supports the discovery made by (Olobatoke and Mulugeta, 2011) that garlic supplementation increased egg weight more than the control group. The increase in egg weight may be related to the presence of organosulfur compounds in garlic and wild leek, which has been reported by Abad et al. (2020) to increase egg weight in laying chickens. All

supplemented treatments increased the rough index, which could confirm that both plants are good sources of pigments. Flavonols, including kaempferol, quercetin, and myricetin, are the most ubiquitous class in allium vegetables (Crozier et al., 2006). Flavonoids are plant secondary metabolites that play an essential role as active pharmaceuticals (Zeng et al., 2017), and they are responsible for the different pigments and aromas (Nuutila et al., 2002). Our study suggests that incorporating garlic supplementation into their diet can enhance their lipid profile, reducing total cholesterol, triglycerides, VLDL, and LDL levels while elevating HDL concentration. Garlic exhibits hypocholesterolemic effects by impeding key enzymes responsible for cholesterol and lipid production, including malic enzyme, fatty acid synthase, glucose-6-phosphate dehydrogenase, and 3-hydroxy-methyl-glutaryl-CoA reductase (Canogullari et al., 2010). On the other hand, some researchers attribute garlic's cholesterol-lowering action to steroidal saponins, which may impede cholesterol absorption in the intestine or directly influence cholesterol metabolism (Sobolewska et al., 2016). Also, Singh and Porter (2006) reported employing compounds having an allyl-disulfide or allyl-sulphydryl group that efficiently suppressed cholesterol production, most likely through the mediation of sterol 4 α -methyl oxidase.

In avian nitrogen metabolism, uric acid serves as the primary end-product, and reducing garlic leaves' effect can confirm garlic's positive effect on the health and optimal function of the kidney. Also, the positive effect of garlic on kidney function and reducing serum uric acid levels was reported by Nasiri et al. (2017) and Ziamajidi et al. (2017). Studies have shown that feeding quails with garlic leaves improved the jejunum and ileum villus height-to-crypt ratio. These results indicated that the inclusion of garlic leaves in the quail diet improved the physiological structure of the intestinal epithelial layer, which in turn enhanced their digestive capacity and resulted in better nutritional absorption and assimilation (Adibmoradi et al., 2006). The result showed that garlic inclusion into quail's diet elevated the digestibility of OM, ash and crude protein. This result agreed with (Olayinka et al., 2022b), who found that adding garlic to Japanese quail diets enhanced digestibility by managing the microbial population, minimizing digestive disturbances, suppressing pathogenic bacteria, and enhancing feed efficiency.

Ultimately, the results lead to the conclusion that the addition of 1% garlic leaves to the quail's diet results in an improvement in egg weight. Adding garlic leaves to the diet has enhanced intestinal morphology and nutrient digestibility. These effects were observed for all levels of garlic leaves but were more evident at the 1% level. Furthermore, including 1% garlic leaves improved lipid profiles and reduced uric acid concentration in the serum. However, both plants were found to be rich sources of pigment and increased the rough index.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution statement

All study conception and design, Material preparation, data collection and analysis were performed by the initial author. Also, the first draft of the manuscript was written by corresponding author and approved the final manuscript.

Data Availability

The datasets generated during the current study are available in the Open Science Framework repository, DOI 10.17605/OSF.IO/RDEYH

Ethics approval

This study was performed in line with the principles of the Declaration of (FASS., 2012). Approval was granted by the Ethics Committee of University of Raparin (15/7/2023: 756-26).

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Tables

Table 1. Composition, calculated and analyzed nutrient contents (%) of experimental diets							
Ingredient	Control	Wild leek			Garlic leaves		
		0.5	1	1.5	0.5	1	1.5
Wild leek	0	0.5	1	1.5	0	0	0
Garlic leaves	0	0	0	0	0.5	1	1.5
Corn	48.04	47.74	47.44	47.15	47.60	47.15	46.70
Soy bean meal	35.55	35.30	35.05	34.80	35.40	35.25	35.09
Soybean oil	5.73	5.76	5.78	5.80	5.81	5.89	5.97
CaCO3	7.87	7.87	7.87	7.87	7.87	7.87	7.87
Dicalcium phosphate	1.80	1.80	1.81	1.81	1.80	1.81	1.81
Common Salt	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Vit Premix ¹	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Mi Premix ²	0.13	0.13	0.13	0.13	0.13	0.13	0.13
DL-Methionine	0.22	0.22	0.23	0.23	0.22	0.23	0.23
L-Lysine	0.09	0.10	0.11	0.12	0.10	0.10	0.11
Threonine	0.09	0.09	0.10	0.10	0.09	0.09	0.10
Calculated analysis							
Crude protein %	20	20	20	20	20	20	20
ME kcal/kg	2900	2900	2900	2900	2900	2900	2900
Calcium %	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Total Phos %	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Avail Phos %	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Cysteine, %	0.31	0.31	0.31	0.30	0.31	0.31	0.30
Lysine, %	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Methionine, %	0.51	0.51	0.52	0.52	0.51	0.51	0.52
TSAA, %	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Na %	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Cl %	0.26	0.26	0.26	0.26	0.26	0.26	0.26
K %	0.86	0.86	0.85	0.84	0.86	0.85	0.85
Analyzed composition							
Dry matter %	90.22	90.58	89.14	90.34	90.56	89.88	90.09
Crude protein %	20.50	20.42	20.57	20.12	20.55	20.28	20.10
Crude fiber %	3.12	3.33	3.51	3.64	3.28	3.44	3.48
Ash %	6.94	7.09	7.32	7.50	6.48	6.08	6.12
¹ Provided per Kg of diet: vitamin A 8800 IU, vitamin D3 2500 IU, Vitamin E 11 IU, Vitamin K3 2.2 IU, Thiamin 1.5 mg, Riboflavin 4 mg, Nicotinic acid 7.85 mg, Pantothenic acid 34.65 mg, Pyridoxin 2.46 mg, folic acid 0.48 mg, Vitamin B124 mg and Antioxidant 400 mg.							

² Provided per Kg of diet: Manganese 74.4 mg, Zinc 64.67 mg, Iron 75 mg, Copper 6 mg, Iodine 0.86 mg, Selenium 0.2 mg and Choline chloride 200 mg

Table 2. Chemical composition of wild leek (*Allium tricoccum*) and garlic (*Allium sativum*) leaves on a DM basis (%).

Plant name	DM	Ash	CP	CF	EE	NEF
Wild leek	91.52	13.92	18.93	22.06	3.28	33.32
Garlic leaves	91.60	9.39	24.36	15.43	2.98	39.43

Abbreviation: DM, Dry matter; CP, Crude protein; CF, Crude fiber; EE, Ether extract; NFE, Nitrogen-free extract.

Table 3. The bioactive components of the Garlic leaves ethanolic extract and their bioactivities

Name of bioactive Compound	Formula	Retention time	Percentage	Bioactivities	References
Isopentyl formate	C ₆ H ₁₂ O ₂	9.222	86.451%	Antioxidant	(Machado et al., 2014)
Diallyl trisulfide	C ₆ H ₁₀ S ₃	11.018	6.516%	Antioxidant	(Kalayarasan et al., 2009)
Allyl methyl trisulfide	C ₄ H ₈ S ₃	8.652	3.978%	Antibacterial and antioxidant	(Wang et al., 2022)
Diallyl disulfide	C ₆ H ₁₀ S ₂	7.390	3.055%	Antioxidant and anti-inflammatory	(Hasan et al., 2020)

Table 4. The bioactive components of the Wild leek ethanolic extract and their bioactivities

Name of bioactive compound	Formula	Retention time	Percentage	Bioactivities	References
Dipalmitin	$C_{35}H_{68}O_5$	23.236	37.30%	Antioxidant and anti-microbial	(Vidya and Jose, 2023)
Chlorozotocin	$C_9H_{16}ClN_3O_7$	6.648	17.93%	Antioxidant	(Li et al., 2020)
Muramic acid	$C_9H_{17}NO_7$	9.276	9.85%	Antioxidant	(Belkacemi et al., 2020)
Tetracontane-1,40-diol	$C_{40}H_{82}O_2$	19.556	7.59%	Antibacterial	(Al-Marzoqi et al., 2016)
2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	7.586	5.88%	Antimicrobial	(Hashem et al., 2019)
Diosgenin	$C_{27}H_{42}O_3$	17.862	3.55%	Antioxidant	(Mohseni-Moghaddam et al., 2023)
Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	15.419	2.84%	Antibacterial	(Hadi and Hussein, 2016)
Ethyl cholate	$C_{26}H_{44}O_5$	16.435	2.67%	Antibacterial	(Moni et al., 2021)
Methyl palmitoleate	$C_{17}H_{32}O_2$	14.927	2.57%	Antibacterial and antioxidant	(Naveen et al., 2021)
2-[3-Cyclohexylaminopropylamino]ethylthiophosphate	$C_{11}H_{25}N_2O_3PS$	12.170	2.42%	Antioxidant	(Tumosienė et al., 2020)
3-Pyridine Propionaldehyde carbethoxy hydrazone	$C_8H_{15}N_3O_2$	13.198	2.37%	Antioxidant	(Kaddouri et al., 2020)
Methionine sulfoxide	$C_5H_{11}NO_3S$	10.897	2.18%	Antioxidant	(Meshkibaf et al., 2019)
D-Fructose, diethyl mercaptal, pentaacetate	$C_{20}H_{32}O_{10}S_2$	12.604	1.51%	Antibacterial	(Hameed et al., 2018)
N-[4-(4-Chlorophenyl)isothiazol-5-yl)-1-methylpiperidin-2-imine	$C_{15}H_{16}ClN_3S$	11.711	1.34%	Antibacterial	(Warda et al., 2022)

Table 5. Effects of wild leek (*Allium tricoccum*) and garlic (*Allium sativum*) leaves supplementation on production performance in laying quails from 30 to 40 of age

Treatments	Egg Production (%)	Egg Weight (g)	Egg Mass (g/quail/day)	ADFI (g)	FCR (g/g)
Control	67.73 ^{ab}	11.72 ^{bc}	7.97 ^{ab}	32.95 ^a	4.24
Garlic leaves 0.5 %	71.57 ^a	11.70 ^{bc}	8.37 ^a	32.64 ^{ab}	3.95
Garlic leaves 1 %	69.54 ^{ab}	11.95 ^a	8.31 ^{ab}	33.01 ^a	4.06
Garlic leaves 1.5 %	65.22 ^b	11.90 ^{ab}	7.76 ^{ab}	31.64 ^b	4.17
Wild leek 0.5 %	67.86 ^{ab}	11.96 ^a	8.10 ^{ab}	32.92 ^a	4.14
Wild leek 1 %	69.10 ^{ab}	11.78 ^{abc}	8.14 ^{ab}	32.78 ^a	4.10
Wild leek 1.5 %	66.38 ^{ab}	11.69 ^c	7.73 ^b	31.99 ^{ab}	4.21
-----Probability-----					
P- value	0.0125	0.0001	0.0128	0.0003	0.0912
SEM ¹	0.49	0.02	0.06	0.1	0.03
Abbreviations: ADFI; Average Daily Feed Intake, FCR; Feed Conversion Ratio.					
¹ Standard Error of Mean.					
^{a-c} Values in the same column and under each main effects with different letters are significantly different (P< 0.05).					

Table 6. Effects of wild leek (*Allium tricoccum*) and garlic (*Allium sativum*) leaves supplementation on internal and external indices of egg quality in laying quails from 30 to 40 of age

Treatments	Egg shape Index	Yolk High (mm)	Albumin High (mm)	Shell Thickness (mm)	Yolk Ratio (%)	Albumin Ratio (%)	Yolk to albumin ratio (%)	Shell Ratio (%)	Haugh Unit	Index (Rouch)
Control	77.7	11.84	5.23	0.234	30.67	61.29	50.26	8.22	93.24	4.00 ^c
Garlic leaves 0.5 %	76.8	11.49	5.17	0.232	31.05	60.61	51.44	8.11	92.75	4.52 ^{ab}
Garlic leaves 1 %	76.5	11.71	5.19	0.228	30.87	61.32	50.87	8.07	92.82	4.34 ^b
Garlic leaves 1.5 %	77.2	11.84	5.17	0.232	30.88	60.60	50.91	8.05	92.66	4.62 ^{ab}
Wild leek 0.5 %	76.5	11.85	5.31	0.232	31.28	60.18	52.22	8.14	93.64	4.65 ^a
Wild leek 1 %	76.5	11.83	5.17	0.232	31.16	60.48	51.27	8.06	92.72	4.41 ^{ab}
Wild leek 1.5 %	77.0	11.75	5.08	0.234	31.15	59.97	51.82	8.19	92.52	4.65 ^a
-----Probability-----										
P- value	0.081	0.0659	0.0731	0.0985	0.765	0.138	0.329	0.455	0.0659	0.0001
SEM	0.114	0.063	0.058	0.0075	0.107	0.157	0.228	0.026	0.191	0.028

¹Standard Error of Mean.

^{a-c}Values in the same column and under each main effects with different letters are significantly different (P< 0.05).

Table 7. Effects of wild leek (*Allium tricoccum*) and garlic (*Allium sativum*) leaves supplementation on serum biochemical traits in laying quails from 30 to 40 of age

Treatments	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	Uric acid (mg/dl)
Control	342 ^a	351 ^a	33.5 ^b	70.1 ^a	239 ^a	5.27 ^a
Garlic leaves 0.5%	306 ^{ab}	289 ^b	38.7 ^b	57.8 ^{ab}	210 ^{ab}	4.72 ^{ab}
Garlic leaves 1%	253 ^b	221 ^c	44.5 ^{ab}	51.4 ^b	164 ^b	4.22 ^b
Garlic leaves 1.5%	324 ^{ab}	285 ^b	54.0 ^a	56.9 ^{ab}	213 ^{ab}	5.10 ^{ab}
Wild leek 0.5%	315 ^{ab}	295 ^{ab}	38.0 ^b	59 ^{ab}	218 ^{ab}	4.77 ^{ab}
Wild leek 1%	286 ^{ab}	303 ^{ab}	44.0 ^{ab}	60.6 ^{ab}	182 ^b	5.12 ^{ab}
Wild leek 1.5%	330 ^{ab}	318 ^{ab}	43.2 ^{ab}	63.6 ^{ab}	223 ^{ab}	5.25 ^{ab}
-----probability-----						
P- value	0.0388	0.0001	0.0003	0.0138	0.0261	0.0358
SEM	8.09	8.26	1.42	1.38	7.25	0.10

Abbreviations: HDL; High Density Lipoprotein, LDL; Low Density Lipoprotein, VLDL; Very Low-Density Lipoprotein.

¹Standard Error of Mean.

^{a-c}Values in the same column and under each main effects with different letters are significantly different (P< 0.05).

Table 8. Effects of wild leek (<i>Allium tricoccum</i>) and garlic (<i>Allium sativum</i>) leaves supplementation on relative weight of gastrointestinal organs to live body weight (%) in laying quails from 30 to 40 of age									
Treatments	Carcass Weight (%)	Liver Weight (%)	Gizzard Weight (%)	Pancreas Weight (%)	Heart Weight (%)	Duodenum Weight (%)	Jejunum Weight (%)	Ileum Weight (%)	Cecum Weight (%)
Control	56.60	4.04	2.47	0.26	0.84	1.75	3.09	1.04	1.46
Garlic leaves 0.5%	58.17	4.00	2.55	0.33	0.98	1.57	2.73	1.08	1.59
Garlic leaves 1%	54.83	2.75	2.44	0.29	0.82	1.72	1.97	0.91	1.08
Garlic leaves 1.5%	55.50	3.34	2.49	0.33	0.80	1.48	3.14	1.99	1.00
Wild leek 0.5%	55.17	2.89	2.47	0.25	0.79	1.66	2.07	0.77	1.06
Wild leek 1%	56.00	3.12	2.49	0.27	0.96	1.55	2.50	0.84	1.23
Wild leek 1.5%	61.00	3.08	2.68	0.24	0.90	1.31	2.18	0.73	1.40
-----Probability-----									
P- value	0.6877	0.133	0.847	0.380	0.0729	0.1165	0.0534	0.167	0.059
SEM	1.00	0.15	0.04	0.01	0.02	0.56	0.13	0.04	0.06
¹ Standard Error of Mean.									
^{a-b} Values in the same column and under each main effects with different letters are significantly different (P< 0.05).									

Table 9. Effects of wild leek (*Allium tricoccum*) and garlic (*Allium sativum*) leaves supplementation on intestinal morphology in laying quails from 30 to 40 of age

Treatments	Jejunum				Ileum			
	Villus high (µm)	Crypt depth (µm)	Villus high / Crypt depth ratio	Villus surface area (mm ²)	Villus high (µm)	Crypt depth (µm)	Villus high / Crypt depth ratio	Villus surface area (mm ²)
Control	585	90.7	6.15 ^b	0.025	493	85.6	5.42 ^b	0.054
Garlic leaves 0.5 %	588	76.7	7.79 ^{ab}	0.026	537	77.7	6.96 ^a	0.068
Garlic leaves 1 %	641	72.1	8.98 ^a	0.028	534	75.2	6.62 ^a	0.069
Garlic leaves 1.5 %	603	84.0	6.89 ^{ab}	0.026	519	86.2	6.04 ^{ab}	0.060
Wild leek 0.5 %	566	84.4	6.83 ^{ab}	0.023	521	83.2	6.36 ^{ab}	0.063
Wild leek 1 %	579	78.5	7.44 ^{ab}	0.028	526	78.5	6.20 ^{ab}	0.069
Wild leek 1.5 %	573	88.4	6.67 ^{ab}	0.026	511	83.5	6.13 ^{ab}	0.060
-----Probability-----								
P- value	0.1210	0.2145	0.0363	0.2873	0.6947	0.5990	0.0237	0.1091
SEM	7.71	2.14	0.25	0.0006	6.76	1.72	0.14	0.0017

¹Standard Error of Mean.

^{a-b} Values in the same column and under each main effects with different letters are significantly different (P< 0.05).

Table 10. Effects of wild leek (*Allium tricoccum*) and garlic (*Allium sativum*) leaves supplementation on apparent ileal digestibility in laying quails from 30 to 40 of age

Treatments	DM	OM	Ash	CP
Control	85.59	66.93 ^b	43.86 ^b	36.35 ^b
Garlic leaves 0.5 %	86.21	70.47 ^{ab}	49.13 ^a	44.58 ^a
Garlic leaves 1 %	88.27	75.63 ^a	56.90 ^a	50.04 ^a
Garlic leaves 1.5 %	87.69	73.83 ^a	56.06 ^{ab}	52.35 ^{ab}
Wild leek 0.5 %	85.83	70.53 ^{ab}	52.42 ^{ab}	48.43 ^{ab}
Wild leek 1 %	87.00	72.06 ^{ab}	51.58 ^{ab}	43.80 ^{ab}
Wild leek 1.5 %	86.69	71.84 ^{ab}	50.32 ^{ab}	49.03 ^{ab}
-----Probability-----				
P- value	0.595	0.0047	0.0363	0.0154
SEM	0.41	0.63	1.15	1.31

Abbreviations: CP; Crude Protein, DM; Dry Matter, OM; Organic Matter.

¹Standard Error of Mean.

^{a-b}Values in the same column and under each main effects with different letters are significantly different (P< 0.05).

Figures

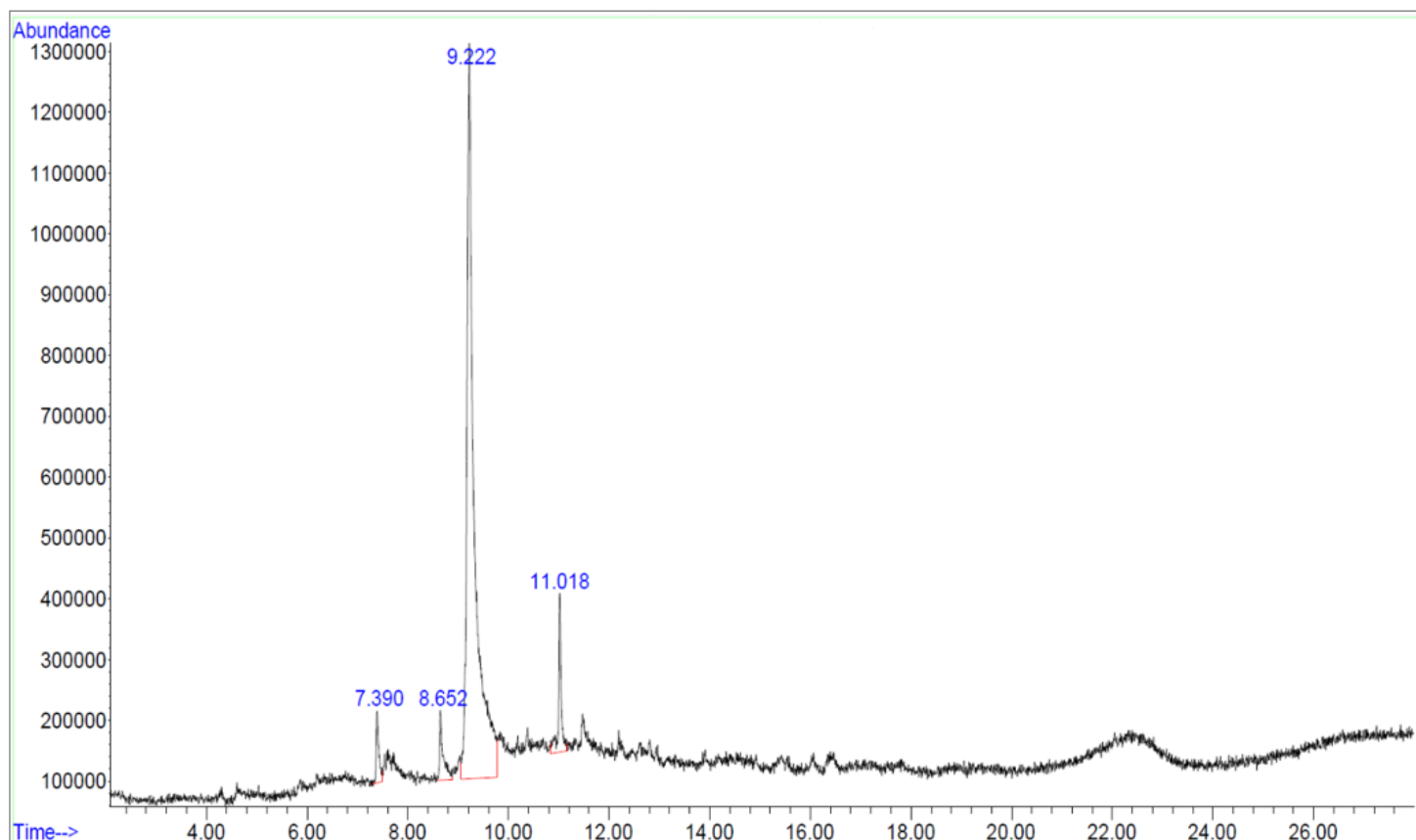


Figure 1

Chromatographic profile (GC-MS) of the Garlic leaves ethanolic extract

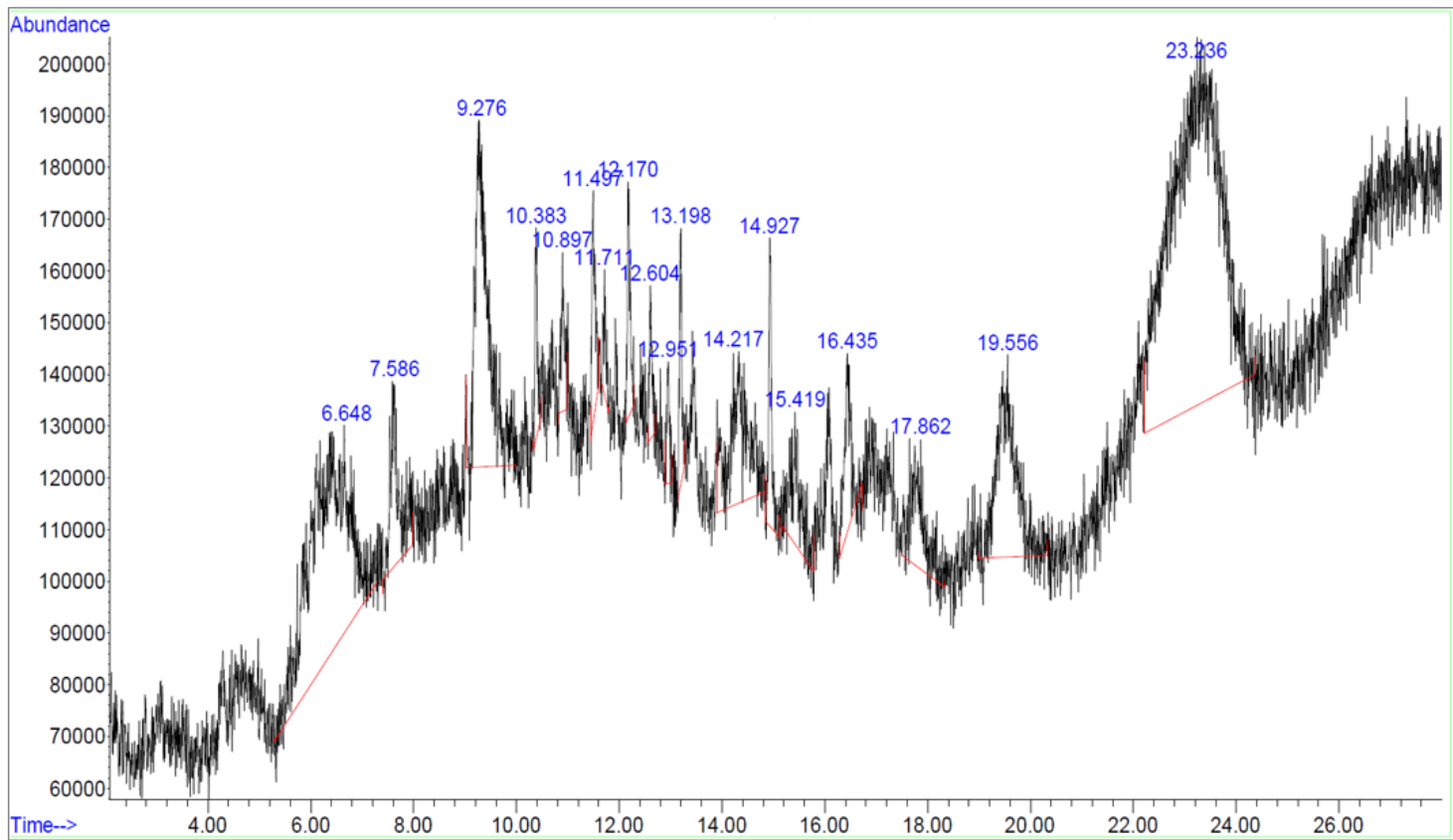


Figure 2

Chromatographic profile (GC-MS) of the Wild leek ethanolic extract