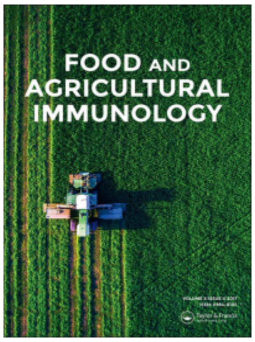

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Effect of anise seed (*Pimpinella anisum* L.) and protease alone or in combination on growth performance, carcase characteristics, humoral immunity, nutrients digestibility and cecal microbiota in Japanese quails

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ABSTRACT

The trial investigated the effects of anise seed powder and protease, alone and combined, in Japanese quail diets. 540 seven-day-old quails were divided into four groups: Control (basal diet), Anise Seed Group (1000 mg/kg anise seed powder), Protease Enzyme Group (30,000 IU/kg *Bacillus subtilis*-derived protease enzyme), and a combination of anise powder and protease. The combination significantly increased weight gain and dressing percentage compared to the control. Quails receiving protease, anise powder, or both showed higher antibody titers against infectious diseases. The population of beneficial Lactobacillus increased in groups receiving protease and the combination. Additionally, the presence of harmful bacteria like *Escherichia coli* and Salmonella decreased significantly in quails supplemented with both protease enzyme and anise powder. In summary, combining protease and anise powder improved growth performance, immune responses, increased Lactobacillus population, and decreased pathogenic bacteria in quails.

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Japanese quails; protease; anise seed powder; growth; microbiota; digestibility

Introduction

In contemporary broiler production, the reliance on antibiotics is often deemed unavoidable owing to their positive impact on broiler health and productivity (Hafeez et al., 2024a; Landy et al., 2020; Saleh et al., 2017). However, the widespread acknowledgment of their adverse effects has prompted significant scrutiny, leading to stringent restrictions on their unbridled use in many developed countries (Abudabos et al., 2018; Ali Tavakkoli

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et al., 2021; Foroutankhah et al., 2019). Consequently, there is an ongoing quest for non-antibiotic alternatives, including herbs, probiotics, organic acids, and other compounds, to sustain broiler growth without imparting adverse effects on end consumers (Foroughi et al., 2011; Hafeez et al., 2024b; Jabbar et al., 2021b; Khan & Naz, 2013; Naz et al., 2024).

Anise (*Pimpinella anisum* L.) has a widespread history of use for enhancing animal and human health. Primarily cultivated in southern Europe and Southeast Asia, its fruits, commonly known as seeds, are the utilisable parts of the plant (Al-Beitawi et al., 2009). These seeds contain 2–6% essential oils, eugenol, estragole, phenolic acids, and trans-anethole, the primary component of the oil (Christaki et al., 2012), known for its potent phytoestrogen properties. Anise has been recognised for its antioxidant, antimicrobial, antibacterial, antipyretic, and antifungal properties over the years. Moreover, the essential oil from anise seeds has been found to boost immunity, aid digestion, and stimulate milk secretion (Frankič et al., 2009). Al-Shammari et al. (2017) found that the highest inclusion levels of anise seed powder had a stimulating effect on the physiological traits of the birds. Cho et al. (2014) concluded that supplementation of plants functional attributes containing star anise improved growth performance, and inhibited *E. coli* and *Clostridium perfringens* proliferation in the intestines of broilers under oral *C. perfringens* challenge. Charal et al. (2017) found that increasing levels of anise oil in the diet of broilers resulted in a linear reduction in growth performance.

Protease, an enzyme specialised in breaking down proteins, is widely employed as a supplement in broiler diets owing to its acknowledged capacity to enhance nutrient digestibility (Jabbar et al., 2021a). Previous research has demonstrated notable benefits, including improved digestibility, enhanced bone strength and reduced abdominal fat in broilers with the addition of exogenous protease enzyme (Hafeez et al., 2020; Jabbar et al., 2021b). According to Fru-Nji et al. (2011), feed additives containing proteases have the potential to complement the functions of endogenous pepsin and pancreatic enzymes. This supplementation occurs by enhancing the hydrolysis and solubilis*ation of* prot*eins, b*oth *in vitro* and *in vivo*. Our hypothesis posited that supplementing a combination of protease and anise powder would enhance growth performance by improving nutrient digestibility and creating a favourable intestinal environment. However, there is limited knowledge regarding the combined effects of proteases and anise powder in poultry. Recently, studies by Hafeez et al. (2020, 2021) highlighted the superior effects of protease on production performance, nutrient digestibility, bone strength, and blood metabolomics in comparison to phytogenic. Importantly, there exists limited research that directly describes the effects of supplementing herbal plants and protease enzyme. Consequently, the present study aims to evaluate and compare the impacts of protease enzyme and anise powder on key parameters such as growth performance, relative organs weight, immune response, nutrients digestibility and cecal microbiota in broiler quails.

Materials and methods

Broiler management

In this study, a total of 540 one-day-old male Japanese quails (average weight 15.6 ± 0.2 g) were randomly assigned to four experimental treatments, each with five replicates.

The control group received a basal diet (Table 1), according to NRC (1994) comprising corn and soybean meal, while the second group received a fine powder supplementation of anise seed powder at a dosage of 1000 mg/kg. Third cohort received supplementation with a protease enzyme at a concentration of 30,000 IU/kg (1500 FTU/kg), sourced from *Bacillus subtilis*. This enzyme's primary function is to break down proteins and proteinaceous anti-nutrient factors found in plant materials, with an activity level of 600,000 units per gram. Fourth group was supplemented with combination of anise seeds powder and protease enzyme. The supplementation period commenced in the second week of the experiment and continued until the fifth week. The basal diet, comprising starter and finisher phases, was provided to the birds, with supplements mixed into the respective groups' basal diets. The quails were raised on the floor with a stocking density of 10.5 birds/m^2. Throughout the brooding phase, the room temperature was initially established at 34 ± 1.4°C and progressively adapted to the standard temperature of 26 ± 1.5°C. The overall duration of the experiment spanned 42 days.

Performance traits

The birds and feed were weighed upon placement at weekly intervals for live performance measurements. Mortality was recorded as it occurred. These measurements were utilised to determine body weight, feed intake, and feed conversion ratio (FCR), with adjustments made for mortality. At the conclusion of the feeding trial, five quails from each replication were slaughtered to assess dressing yield. The warm weight of the dressed chicken was measured after slaughter and evisceration. The dressing percentage, calculated as the ratio of the dressed weight to the live weight of the birds, was then computed and recorded (Abedpour et al., 2017; Shariat Zadeh et al., 2020).

Table 1. Feed composition of Japanese quails.

Percent constituents	Starter phase (0–21 days)	Finisher phase (22–35 days)
Maize (yellow)	57	59
Soybean meal (48%)	38	28
Limestone	1.5	1.6
Dicalcium phosphate	1.6	1.7
Salt (NaCl)	0.3	0.3
DL-Methionine	0.16	0.11
Vitamins and minerals premixes	0.50	0.50
Chemical analysis		
Protein (%)	22.0	21.0
Metabolisable energy (Kcal/kg)	2800	2900
Crude fibre (%)	3.0	3.5
Lysine (%)	1.3	1.3
Calcium (%)	0.95	0.85
Methionine + Cysteine (%)	0.12	0.08
Threonine (%)	0.9	0.6
Available Phosphorus (%)	0.45	0.45
Sodium (%)	0.02	0.02
Chloride (%)	0.04	0.04

Relative organs weight

At the end of the experiment, 25 quails per treatment (5 per replicate pen) were slaughtered. Skilled personnel removed and weighed organs, including the liver, spleen, bursa of Fabricius, thymus, heart, and gall bladder. The relative organ weight, expressed as a percentage of live body weight, was then calculated.

Antigen–antibody titre evaluation

Antibody titres for Infectious Bronchitis (IB), Newcastle disease (ND), and Infectious Bursal Disease (IBD) were assessed using the Hemagglutination Inhibition (HI) test. On day 35, blood samples were collected, and serum was isolated through centrifugation. The HI test, conducted in a microtitration plate, involved twofold dilution of serum samples and the addition of normal saline, antigen, and chicken RBCs to each well. After incubation, antigen and antibody titres were confirmed, providing insights into the immune response against the specified diseases.

Assessment of parameters for nutrient digestibility

To assess nutrient digestibility, four birds per replicate were transferred to individual experimental cages to collect faecal samples on day 35 of the trial. The gathered samples were then analysed for Dry Matter (DM), Ash, Crude Protein (CP), Crude Fibre (CF), and Ether Extract (EE) using standard procedures.

Digestibility of apparent metabolisable energy

To quantify the Apparent Metabolisable Energy (AME), a bomb calorimeter (BC) standardised with benzoic acid was employed. The formula below was utilised to estimate the apparent metabolisable energy on a dry matter basis:

$$\text{AME} = (\text{Energy consumption} - \text{Energy lost}) \div \text{Feed consumption}$$

Cecal microbiota

Cecal digesta samples were collected from 10 birds in each dietary group using a sterile collection tube and subsequently stored at −20°C for microbiota colonisation assessment (Alharthi et al., 2022). The colonies, ranging from 50 to 300, were visibly distinct and easily countable. A total of 10 μL of the samples were cultured on specific media corresponding to the bacterial species under investigation. Lactobacillus spp. were cultured on MRS agar, while Salmonella spp. and Escherichia coli were cultured on EMB. Colony counting was performed using a colony counter.

Statistical analysis

Statistical analysis of data for all parameters was conducted utilising General Linear Model (SAS software), employing a one-way analysis of variance with a completely randomised design. The differentiation of means was established through Tukey's honest significant difference test.

Results

The impact of anise powder and enzyme, either individually or in combination, on the feed intake of Japanese quail is presented in Table 2. The results revealed no significant difference in feed intake between the control and experimental groups.

Table 2 displays the effect of anise powder and enzyme, either individually or in combination, on the weight gain of Japanese quail. The findings indicated that weekly and overall weight gain was significantly higher ($P < 0.05$) in quails supplemented with both anise powder and enzyme compared to the control in week 3and week 5. However, in week 4 and overall mean weight gain was significantly ($P < 0.05$) higher in anise powder and protease supplementation compared to anise powder.

In Table 2, the effect of protease and anise powder, either individually or in combination, on the weekly and overall feed conversion ratio (FCR) of Japanese quail is depicted. The results demonstrated that the mean weekly and overall FCR did not vary significantly in quails supplemented with both anise powder and protease compared to the control.

Table 3 illustrates the effect of protease and anise powder, either individually or in combination on mean dressing percentage and mortality of Japanese quail. The mean dressing percentage was significantly ($P < 0.05$) higher in the combined anise and protease supplemented group or protease supplemented quails alone compared to the control and anise powder, while mortality was not significantly different in the birds supplemented with supplemented groups compared to the control.

The impact of protease and anise powder, either individually or in combination, on mean relative organ weight is presented in Table 4. The results showed that the mean

Table 2. Effect of protease and anise powder individually or in combination on weekly and overall feed intake of Japanese quail.

	Weekly feed intake of quail (g)				
Group	WK-2	WK-3	WK-4	WK-5	Overall mean
Anise powder	61.4 ± 0.29	81.4 ± 1.18	104.7 ± 0.46	132.3 ± 0.28	158.4 ± 0.70
Protease	60.7 ± 0.36	81.2 ± 0.59	102.8 ± 1.36	132.0 ± 0.23	157.2 ± 0.21
Anise powder + protease	61.1 ± 0.64	79.4 ± 1.01	103.1 ± 0.24	131.7 ± 0.10	156.8 ± 0.11
Control	59.3 ± 0.60	81.4 ± 1.15	102.8 ± 0.51	131.9 ± 0.10	156.7 ± 0.17
P-value	0.43	0.56	0.91	0.88	0.12
weight gain (g)					
Anise powder	21.4 ± 0.43	36.4^{b} ± 0.52	47.8^{b} ± 0.39	51.1^{b} ± 0.84	52.4^{b} ± 0.45
Protease	22.5 ± 0.41	38.8^{b} ± 0.41	49.3^{ab} ± 0.42	52.2^{b} ± 0.81	53.5^{ab} ± 0.49
Anise powder + protease	22.8 ± 0.41	39.6^{a} ± .48	52.3^{a} ± 0.41	53.2^{a} ± 0.43	54.8^{a} ± 0.39
Control	22.4 ± 0.37	38.7^{b} ± 0.39	50.3^{ab} ± 0.41	52.1^{b} ± 0.16	53.8^{ab} ± 0.30
P-value	NS	0.04	0.03	0.04	0.02
feed conversion ratio					
Anise powder	2.86 ± 0.04	2.23 ± 0.06	2.18 ± 0.02	2.58 ± 0.04	03.0 ± 0.02
Protease	2.69 ± 0.04	2.08 ± 0.02	2.08 ± 0.03	2.52 ± 0.04	2.93 ± 0.03
Anise powder + protease	2.67 ± 0.06	2.00 ± 0.04	1.97 ± 0.02	2.47 ± 0.01	2.86 ± 0.02
Control	2.64 ± 0.04	2.10 ± 0.02	2.04 ± 0.01	2.52 ± 0.03	2.91 ± 0.02
P-value	NS	NS	NS	NS	NS

Means with dissimilar superscripts in similar column significantly different at ($P < 0.05$).

Table 3. Effect of protease and anise powder individually or in combination on weekly and overall mean dressing percentage and mortality of Japanese quail.

	Dressing percentage	Mortality (%)
Anise powder	58.0 ± 0.32[b]	1.25 ± 0.04
Protease	59.1 ± 0.24[a]	1.35 ± 0.05
Anise powder + protease	59.6 ± 0.44[a]	1.75 ± 0.02
Control	57.9 ± 0.51[b]	1.50 ± 0.00
P-value	0.023	NS

Note: Mean values bearing different superscripts in a column differ significantly (P<0.05). NS: non-significant.

relative weight of different organs did not change between the control and the experimental groups.

The impact of protease and anise powder, either individually or in combination, on the antibody titre of Japanese quail is presented in Table 5. The results showed that the mean antibody titre against IB, ND and IBD was significantly higher ($P < 0.05$) in quails receiving individual treatments of protease, anise powder or combination of both supplementations.

Table 6 displays the effect of protease and anise powder, either individually or in combination, on the digestibility of DM, CP, EE, CF, NFE, and ash in Japanese quail. The results revealed that these parameters did not vary significantly between the control and the treatment groups.

Table 7 outlines the effect of protease, either individually or in combination, on cecal microbiota and apparent metabolisable energy (AME) of Japanese quail. The results showed that AME did not change significantly ($P < 0.05$) in the groups receiving enzyme and the combination of enzyme with anise powder supplementation compared

Table 4. Effect of anise powder and protease either alone or in combination on mean relative organ weight (%) of quails.

	Bursa	Spleen	Thymus	Heart	Gall bladder	Liver	Gizzard	Intestine
Anise powder	0.32 ± 0.04	0.31 ± 0.01	0.45 ± 0.05	1.4 ± 004	0.30 ± 0.01	4.2 ± 0.07	2.5 ± 0.01	8.09 ± 0.02
Protease	0.33 ± 0.01	0.32 ± 0.00	0.43 ± 0.03	1.4 ± 0.03	0.26 ± 0.00	3.7 ± 0.03	2.6 ± 0.05	8.03 ± 0.09
Anise powder + protease	0.27 ± 0.03	0.30 ± 0.02	0.46 ± 0.02	1.4 ± 0.09	0.28 ± 0.02	3.6 ± 0.01	2.6 ± 0.06	8.01 ± 0.03
Control	0.31 ± 0.02	0.31 ± 0.03	0.43 ± 0.01	1.4 ± 0.12	0.27 ± 0.03	3.5 ± 0.04	2.6 ± 0.08	8.06 ± 0.01
P-Value	NS	NS	NS	NS	NS	NS	NS	NS

NS: Non-significant difference.

Table 5. Effect of protease and protease individually or in combination on antibody titre against different viral vaccination of Japanese quail.

	Antibody titre against vaccination		
Groups	IB	IBD	ND
Anise powder	4.90[a] ± 0.94	4.99[a] ± 0.04	4.76[a] ± 0.36
Protease	4.88[a] ± 0.07	4.95[a] ± 0.02	4.99[a] ± 0.04
Anise powder + protease	4.95[a] ± 0.15	5.03[a] ± 0.03	4.89[a] ± 0.27
Control	4.20[b] ± 0.20	4.32[b] ± 0.03	4.15[b] ± 0.29
P-value	0.02	0.03	0.02

Means with dissimilar superscripts in similar column differ significantly at ($P < 0.05$).
IB: infectious bronchitis; IBD: infectious bursal disease; ND: New Castle disease.

Table 6. Effect of protease and anise powder individually or in combination on nutrients digestibility of Japanese quail.

Group	Digestibility coefficient (%)					
	Dry matter	Crude protein	Ether extract	Crude fat	Nitrogen free extract	Ash
Anise powder	70.40 ± 1.3	68.08 ± 0.83	66.43 ± 0.79	64.86 ± 0.62	52.61 ± 0.29	28.26 ± 0.31
Protease	70.96 ± 1.57	69.82 ± 1.17	66.97 ± 1.13	64.83 ± 0.38	53.73 ± 1.30	28.19 ± 0.46
Anise powder + protease	71.25 ± 1.94	69.43 ± 1.21	67.51 ± 1.73	64.78 ± 0.68	53.75 ± 0.39	28.54 ± 0.10
Control	70.97 ± 1.68	69.61 ± 0.91	67.03 ± 0.87	65.11 ± 0.94	52.62 ± 0.70	28.058 ± 0.32
P-value	NS	NS	NS	NS	NS	NS

Means with different superscripts in column differ significantly at ($P < 0.05$).

Table 7. Effect of protease and protease individually or in combination on AME and cecal microbiota of Japanese quail.

Group	Apparent metabolisable energy (AME) (MJ/kg)	Gut microbiota ($\log_{10}$ cfu/g)		
		Lactobacillus	*E. coli*	*Salmonella*
Anise powder	13.4 ± 0.01	$2.53^{ab} \pm 0.52$	$3.12^{ab} \pm 0.33$	$3.12^{b} \pm 0.32$
Protease	13.5 ± 0.02	$2.56^{ab} \pm 0.51$	$3.44^{ab} \pm 0.12$	$3.45^{ab} \pm 0.78$
Anise powder + protease	13.8 ± 0.04	$3.33^{a} \pm 1.2$	$2.75^{b} \pm 0.67$	$2.77^{b} \pm 0.47$
Control	13.6 ± 0.03	$1.23^{b} \pm 0.25$	$4.01^{a} \pm 0.02$	$4.17^{a} \pm 0.20$
P-value	0.021	0.02	0.04	0.02

Means with different superscripts within a column differ significantly at ($P < 0.05$).

to the control. Furthermore, the population of *Lactobacillus* increased significantly ($P <$ 0.05) in the groups receiving protease and the combination of protease and anise powder supplementation. Additionally, the populations of *E. coli* and *Salmonella* in the ceca decreased significantly ($P < 0.05$) in quails supplemented with both protease enzyme and anise powder.

Discussion

In this study, quails supplemented with protease and anise powder exhibited higher body weight gain, consistent with prior research on the positive impact of anise supplementation in broiler chicks. Previous studies, such as Al-Kassie (2008) and Muhammad et al. (2014), demonstrated improved bird performance with anise addition. Mohammed (2019) reported enhanced performance based on LBW and BWG with anise inclusion. Additionally, Elkomy et al. (2023) found that combined supplementation of anise and grape seed oil increased body weight gain in Japanese quails with no effect on feed intake. The active ingredients in anise, including anethole, anisaldehyde, eugenol, methyl chavicol and estragole, may contribute to this increase. Notably, anethole and eugenol have digestive stimulating effects, influencing live weight gain and feed conversion. Anise essential oil has also been associated with antimicrobial, antifungal, and antiparasitic properties in various studies (Çabuk et al., 2003; Singh et al., 2002; Soliman & Badea, 2002; Tabanca et al., 2003). Furthermore, according to Jamroz and Kamel (2002), it improved protein, cellulose, and lipid digestion. Additionally, Hernandez et al. (2004) found that supplementation enhanced nutrient absorption digestibility in both the whole-tract and ileal regions.

Additionally, it increased the impacts of pancreatic lipase and amylase, as observed in the study by Ramakrishna et al. (2003). Throughout the experimental period, the inclusion of anise powder, protease, or their combination in quail chicks' diet did not influence feed intake. All treated groups exhibited nearly identical feed consumption levels. Consistent with our findings, similar non-significant variations in quail feed consumption were observed in response to different levels of anise seeds in previous studies. No previous studies have documented the mixture of anise and protease supplementation in poultry. In the present study, the combination of protease and anise powder demonstrated synergistic effects on weight gain in quails. The positive impact of protease is likely attributed to reduced antinutritional factors, enhanced protein digestibility, and modifications to intestinal microflora. The increased digestibility observed in broilers fed herbal-plant supplements could be linked to the stimulation of digestive enzyme secretion prompted by the supplementation. Furthermore, the improved bone measurements and histological features may also result from enhanced digestibility indices in broilers fed with protease enzyme.

The observed increase in body weight in groups treated with anise powder and protease, compared to the control group, was accompanied by a rise in their relative carcase weight. This outcome may be attributed to the enhancement of nutrient digestibility and absorption in the digestive tract, leading to increased protein deposition in tissues. Contrary to our results, prior research has suggested a beneficial impact of incorporating aniseed powder into the broiler's diet. Previous studies by Muhammad et al. (2014) and Mohammed (2019) reported that such supplementation resulted in increased carcase weight and higher dressing percentages compared to the control group. However, in the current study, such positive outcomes were only observed when protease was supplemented along with anise powder. While no existing studies specifically address the synergistic effects of protease and anise seed powder on poultry production performance, our current research suggests that the enhanced carcase quality observed in quails fed a combination of protease and anise may be attributed to increased nutrient retention. In the current study, there were no significant changes in relative organ weights between the control and treatment groups. This aligns with the findings of Yazdi et al. (2014), who observed no significant differences in the weights of most relative organs. Conversely, Hamodi and Firas (2011) reported a significant increase in the relative weights of thigh and breast organs in broilers supplemented with anise seeds. Elkomy et al. (2023) found an increase in carcase weight, fat, bursa, and thymus, while the relative weights of spleen, liver, and gizzard did not significantly change in Japanese quails. Variation in the results of relative organ weights has been reported by different authors for broilers (Elkomy et al., 2023; Hernandez et al., 2004; Simsek et al., 2007; Yazdi et al., 2014).

In the current study, humoral response against IB, IBD and ND was significantly higher in the anise, protease and combination of anise and protease supplementation in Japanese quails compared to the control. In the study of Yazdi et al. (2014) antibody titre against avian influenza increased in the group treated with 1 or 10 g/kg diet. As anise has been reported for having antioxidant, antifungal and antimicrobial effects, an increase response of humoral response was anticipated. Our results are in agreement with that of Al-Beitawi et al. (2009) who reported higher antibody production against IBD, NDV and IB in broilers supplemented with anise, black seed, and thyme. Unfortunately, no other reports are available on the effects of anise seeds on poultry immunity.

The intestinal microbiota in livestock and poultry is crucial for maintaining health and promoting growth performance (Wang et al., 2017). In chickens, the microbial community in the ileum is predominantly composed of Lactobacillus spp. In our present study, the addition of protease and anise to the quail diet led to an increased population of *Lactobacillus* and a reduction in *E. coli* and *Salmonella*. This suggests that the inclusion of anise and protease in the quail diet improved the environmental conditions in the quail caecum, resulting in a significant increase in *Lactobacillus* count. These findings indicate that the supplementation of protease along with anise contributed to an enhanced population of beneficial bacteria and a reduction in harmful pathogens. Anhê et al. (2015) noted that polyphenols from various plants, including anise oil and its primary component anethole, could positively influence gut microbiota by increasing Lactobacillus spp. Gülçin et al. (2003) and Wang et al. (2011) highlighted the antibacterial properties of anise oil, particularly against aerobic and anaerobic bacteria. Elkomy et al. (2023) reported results similar to ours, showing increased *Lactobacillus* numbers and decreased *E. coli* and *Salmonella* in response to anise supplementation. Saleh et al. (2014) found that linalool, a component of anise, inhibited the growth of various pathogenic bacteria in broilers, supporting our findings.

Conclusion

In summary, the present study's findings support the conclusion that the combination of protease and anise powder in the diet led to enhanced growth performance and improved immune response in Japanese quails. Additionally, there was a significant increase in the cecal population of *Lactobacillus*, a beneficial bacteria, and a notable decrease in pathogenic bacteria such as *E. coli* and *Salmonella* in quails supplemented with the combination of protease and anise seed powder. This suggests the potential synergistic effects of combining protease and anise powder as dietary supplements for optimising the health and performance of quails.

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Disclosure statement

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

Ethical statement

This study has been approved by the departmental committee on ethics and animal welfare the University of Agriculture, Peshawar (23/PS/2022)

References

Abedpour, A., Jalali, S. M. A., & Kheiri, F. (2017). Effect of vegetable oil source and l-carnitine supplements on growth performance, carcass characteristics and blood biochemical parameters of Japanese quails (Coturnix japonica). *Iranian Journal of Applied Animal Science*, *7*(1), 147–153.

Abudabos, A. M., Alyemni, A. H., Dafalla, Y. M., & Khan, R. U. (2018). The effect of phytogenics on growth traits, blood biochemical and intestinal histology in broiler chickens exposed to Clostridium perfringens challenge. *Journal of Applied Animal Research*, *46*(1), 691–695. https://doi.org/10.1080/09712119.2017.1383258

Al-Beitawi, N. A., El-Ghousein, S. S., & Abdullah, H. N. (2009). Antibiotic growth promoters and anise seeds in broiler diets. *Jordan Journal of Agricultural Sciences*, *5*(4), 472–481.

Alharthi, A. S., Al-Baadani, H. H., Abdelrahman, M. M., Alhidary, I. A. (2022). Effects of feeding different levels of sprouted barley on fermentation characteristics, bacterial quantification, and rumen morphology of growing lambs. *Veterinary Sciences*, *10*(1), 15.

Ali Tavakkoli, A., Mirakzehi, M. T., Saleh, H., & Yousefi, M. (2021). The effects of supplementation of Withania coagulans and α-tocopherol acetate in diets containing oxidised oil on growth performance, immune response and antioxidant indices in broiler chickens. *Archives of Animal Nutrition*, *75*(4), 278–293. https://doi.org/10.1080/1745039X.2021.1942765

Al-Kassie, G. A. M. (2008). The effect of anise and rosemary on broiler performance. *International Journal of Poultry Science*, *7*(3), 243–245. https://doi.org/10.3923/ijps.2008.243.245

Al-Shammari, K. I. A., Batkowska, J., & Gryzińska, M. M. (2017). Effect of various concentrations of an anise seed powder (Pimpinella Anisum L.) supplement on selected hematological and biochemical parameters of broiler chickens. *Brazilian Journal of Poultry Science*, *19*(1), 41–46. https://doi.org/10.1590/1806-9061-2016-0331

Anhê, F. F., Varin, T. V., Le Barz, M., Desjardins, Y., Levy, E., & Roy, D. (2015). Gut microbiota dysbiosis in obesity-linked metabolic diseases and prebiotic potential of polyphenol-rich extracts. *Current Obesity Reports*, *4*(4), 389–400. https://doi.org/10.1007/s13679-015-0172-9

Çabuk, M., Alcicek, A., Bozkurt, M., & Imre, N. (2003). Anti-microbial properties of the essential oils isolated from aromatic plants and using possibility as alternative feed additives. In *Proceedings of the 2nd National Animal Nutrition Congress*, 8–20 September; Konya, Turquia (pp. 184–187).

Charal, J. W., Bidner, T. D., Southern, L. L., Janes, M. E., Gutierrez, M. E., & Lavergne, T. A. (2017). Anise oil dosage and its effect on growth performance and jejunal lesions during a Clostridium perfringens challenge in battery trials, and growth performance in a floor pen trial. *Journal of Applied Poultry Research*, *26*(2), 240–252. https://doi.org/10.3382/japr/pfw068

Cho, J. H., Kim, H. J., & Kim, I. H. (2014). Effects of phytogenic feed additive on growth performance, digestibility, blood metabolites, intestinal microbiota, meat color and relative organ weight after oral challenge with Clostridium perfringens in broilers. *Livestock Science*, *160*, 82–88. https://doi.org/10.1016/j.livsci.2013.11.006

Christaki, E., Bonos, E., Giannenas, I., & Florou-Paneri, P. (2012). Aromatic plants as a source of bioactive compounds. *Agriculture*, *2*(3), 228–243. https://doi.org/10.3390/agriculture2030228

Elkomy, A. E., El-Saadany, A. S., Shreif, E. Y., Bayoumi, A. A., El-Maged, A., Alagawany, M. H., Saleh, M., Cho, A. A., Kim, S., & Eltahan, H. M. (2023). Anise and grape seed oils as a feed additive to improve the performance, immune response, and antioxidant activity and reduce caecal pathogenic microbes of quail. *Archives Animal Breeding*, *66*(4), 379–390. https://doi.org/10.5194/aab-66-379-2023

Foroughi, A. R., Torghabeh, H. M., & Saleh, H. (2011). The effect of essential oil of thyme (Thimus vulgaris) on to performance and humoral immune response broilers chicken. *Agricultural Journal*, *6*(6), 299–302. https://doi.org/10.3923/aj.2011.299.302

Foroutankhah, M., Toghyani, M., & Landy, N. (2019). Evaluation of Calendula officinalis L.(marigold) flower as a natural growth promoter in comparison with an antibiotic growth promoter on growth performance, carcass traits and humoral immune responses of broilers. *Animal Nutrition*, *5*(3), 314–318. https://doi.org/10.1016/j.aninu.2019.04.002

Frankič, T., Voljč, M., Salobir, J., & Rezar, V. (2009). Use of herbs and spices and their extracts in animal nutrition. *Acta Argiculturae Slovenica*, *94*(2), 95–102.

Fru-Nji, F., Kluenter, A. M., Fischer, M., & Pontoppidan, K. (2011). A feed serine protease improves broiler performance and increases protein and energy digestibility. *The Journal of Poultry Science*, *48*(4), 239–246. https://doi.org/10.2141/jpsa.011035

Gülçin, I., Oktay, M., Kirecci, E., & Kufrevioglu, O. I. (2003). Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extract. *Food Chemistry*, *83*(3), 371–382. https://doi.org/10.1016/S0308-8146(03)00098-0

Hafeez, A., Ali, S. S., Akhtar, J., Naz, S., Alrefai, A. F., Albeshr, M. F., Israr, M., & Khan, R. U. (2024a). Impact of coriander (*Coriandrum sativum*), garlic (*Allium sativum*), fenugreek (*Trigonella foenum-graecum*) on zootechnical performance, carcass quality, blood metabolites and nutrient digestibility in broilers chickens. *Veterinary Quarterly*, *44*(1), 1–7. https://doi.org/10.1080/01652176.2023.2300948

Hafeez, A., Iqbal, S., Sikandar, A., Din, S., Khan, I., Ashraf, S., Khan, R. U., Laudadio, V., & Tufarelli, V. (2021). Feeding of phytobiotics and exogenous protease in broilers: Comparative effect on nutrient digestibility, bone strength and gut morphology. *Agriculture*, *11*(3), 228. https://doi.org/10.3390/agriculture11030228

Hafeez, A., Khan, D., Naz, S., Alonaizan, R., Al-Akeel, R. K., Israr, M., & Khan, R. U. (2024b). Effect of *Azolla pinnata* meal on growth, immunity, faecal *E. coli*, antioxidant capacity and gut histomorphology in Japanese quails. *Journal of Applied Animal Research*, *52*(1), 2310750. https://doi.org/10.1080/09712119.2024.2310750

Hafeez, A., Shah, S. A. A., Khan, R. U., Ullah, Q., & Naz, S. (2020). Effect of diet supplemented with phytogenics and protease enzyme on performance, serum biochemistry and muscle histomorphology in broilers. *Journal of Applied Animal Research*, *48*(1), 326–330. https://doi.org/10.1080/09712119.2020.1789648

Hamodi, S. J., & Firas, A.-M. K. (2011). Compared between anise seeds (Pimpinella anisum L.) and Roselle flowers (hibiscussabdariffa) by their affected on production performance of broiler. *Advances in Environmental Biology*, *5*, 461–465.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J., & Megias, M. D. (2004). Influence of two plant extract on broiler performance, digestibility, and digestive organ size. *Poultry Science*, *83*(2), 169–174. https://doi.org/10.1093/ps/83.2.169

Jabbar, A., Tahir, M., Alhidary, M. A., Abdelrahman, M., Albaadani, H., Khan, R. U., Selvaggi, M., Laudadio, V., & Tufarelli, V. (2021b). Impact of microbial protease enzyme and dietary crude protein levels on growth and nutrients digestibility in broilers over 15 to 28 days. *Animals*, *11*(9), 2499. https://doi.org/10.3390/ani11092499

Jabbar, A., Tahir, M., Khan, R. U., & Ahmad, N. (2021a). Interactive effect of exogenous protease enzyme and dietary crude protein levels on growth and digestibility indices in broiler chickens during the starter phase. *Tropical Animal Health and Production*, *53*(1), 23. https://doi.org/10.1007/s11250-020-02466-5

Jamroz, D., & Kamel, C. (2002). Plant extracts enhance broiler performance. In non-ruminant nutrition: Anti-microbial agents and plant extracts on immunity, health and performance. *Journal of Animal Science*, *80*(1), 41–46.

Khan, R. U., & Naz, S. (2013). Applications of probiotics in poultry production. *World's Poultry Science Journal*, *69*(3), 621–632. https://doi.org/10.1017/S0043933913000627

Landy, N., Kheiri, F., & Faghani, M. (2020). Evaluation of cottonseed bioactive peptides on growth performance, carcase traits, immunity, total antioxidant activity of serum and intestinal morphology in broiler chickens. *Italian Journal of Animal Science*, *19*(1), 1375–1386. https://doi.org/10.1080/1828051X.2020.1844085

Mohammed, H. N. (2019). Broiler performance response to anise seed powder supplementation. *Iraqi Journal of Veterinary Sciences*, *33*(1), 131–135. https://doi.org/10.33899/ijvs.2019.125533.1055

Muhammad, S. M., Ahmed, M. F., Hussin, I., Abbas, R. Z., Khan, A., & Rafiq, A. (2014). Growth promoting effect of Pimpinella anisum (Aniseed) in broiler chickens. *Boletin Latinoamericano y del Caribe de plantas Medicinales y Aromaticas*, *13*(3), 278–284.

Naz, S., Bibi, G., Nadeem, R., Alhidary, I. A., Dai, S., Israr, M., & Khan, R. U. (2024). Evaluation of biological selenium nanoparticles on growth performance, histopathology of vital organs and genotoxicity in Japanese quails (*Coturnix coturnix japonica*). *Veterinary Quarterly*, *44*(1), 1–10. https://doi.org/10.1080/01652176.2024.2319830

NRC (National Research Council). (1994). *Nutrient requirements of poultry* (9th revised ed.). The National Academies Press. https://doi.org/10.17226/2114

Ramakrishna, R. R., Platel, K., & Srinivasan, K. (2003). In vitro influence of species and specie-active principles on digestive enzymes of rat pancreas and small intestine. *Nahrung*, *47*(6), 408–412. https://doi.org/10.1002/food.200390091

Saleh, N., Allam, T., El-Latif, A.A., & Ghazy, E. (2014). The effects of dietary supplementation of different levels of thyme (Thymus vulgaris) and ginger (Zingiber officinale) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. *Global Veterinaria*, *12*(6), 736–744.

Saleh, H., Golian, A., Kermanshahi, H., & Mirakzehi, M. T. (2017). Effects of dietary α-tocopherol acetate, pomegranate peel, and pomegranate peel extract on phenolic content, fatty acid composition, and meat quality of broiler chickens. *Journal of Applied Animal Research*, *45*(1), 629–636. https://doi.org/10.1080/09712119.2016.1248841

Shariat Zadeh, Z., Kheiri, F., & Faghani, M. (2020). Productive performance, egg-related indices, blood profiles, and interferon-Ɣ gene expression of laying Japanese quails fed on Tenebrio molitor larva meal as a replacement for fish meal. *Italian Journal of Animal Science*, *19*(1), 274–281. https://doi.org/10.1080/1828051X.2020.1722970

Simsek, U. G., Ciftci, M., Dalkilic, B., Guler, T., & Ertas, O. N. (2007). The effects of dietary antibiotic and anise oil supplementation on body weight, carcass characteristics and organoleptic analysis of meat in broilers. *Revue de Médecine Vétérinaire*, *158*(8), 514–518.

Singh, G., Kapoor, I. P., Pandey, S. K., Singh, U. K., & Singh, R. K. (2002). Studies on essential oils, part 10; antibacterial activity of volatile oils of some spices. *Phytotherapy Research*, *16*(7), 680–682. https://doi.org/10.1002/ptr.951

Soliman, K. M., & Badea, R. I. (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food and Chemical Toxicology*, *40*(11), 1669–1675. https://doi.org/10.1016/S0278-6915(02)00120-5

Tabanca, N., Bedir, E., Kirimer, N., Baser, K. H., Khan, S. I., Jacob, M. R., & Khan, I. A. (2003). Anti-microbial compounds from Pimpinella species growing in Turkey. *Planta Medica*, *69*(10), 933–938. https://doi.org/10.1055/s-2003-45103

Wang, G. W., Hu, W. T., Huang, B. K., & Qin, L. P. (2011). Illicium verum: A review on its botany, traditional use, chemistry and pharmacology. *Journal of Ethnopharmacology*, *136*(1), 10–20. https://doi.org/10.1016/j.jep.2011.04.051

Wang, Y., Deng, Q., Song, D., Wang, W., Zhou, H., Wang, L., & Li, A. (2017). Effects of fermented cottonseed meal on growth performance, serum biochemical parameters, immune functions, antioxidative abilities, and cecal microflora in broilers. *Food and Agricultural Immunology*, *28*(4), 725–738. https://doi.org/10.1080/09540105.2017.1311308

Yazdi, F. F., Ghalamkari, G., Toghiani, M., Modaresi, M., & Landy, N. (2014). Anise seed (Pimpinella anisum L.) as an alternative to antibiotic growth promoters on performance, carcass traits and immune responses in broiler chicks. *Asian Pacific Journal of Tropical Disease*, *4*(6), 447–451. https://doi.org/10.1016/S2222-1808(14)60604-6