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EVALUATION OF IN OVO CINNAMON, GINGER OR ANISE EXTRACT INJECTION ON BROILER HATCHING PERFORMANCE

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Abstract: Firstly, this study aimed to evaluate the effects on hatchability and relative weights of yolk, metabolic organ (heart, liver, breast muscle, and thigh muscle) and total digestive system (GUT) of in ovo feeding of cinnamon, ginger or anise extract. Secondly, it was investigated to determine the appropriate dose of cinnamon, ginger or anise extract in in ovo feeding. For this purpose, 480 fertilized broiler eggs were randomly distributed into 12 groups with four replicates as a factorial arrangement of three extracts (cinnamon, ginger, anise) x 4 doses (0, 3, 9, 12 mg/egg) to hatching trays. On the 18th day of incubation, 1 ml of 0.9% saline solution containing 0, 3, 9, 12 mg of cinnamon, ginger or anise extract was injected into the eggs with a 19 mm and 27-gauge needle. The cinnamon and ginger groups had higher hatchability, chick quality and proventriculus weight, while the anise group had higher thigh muscle weight. Chick weight was 12 mg/egg, chick quality was 0 and 3 mg/egg, breast muscle weight was 9 mg/egg, and liver, gizzard and GUT weights were higher at 0, 3 and 9 mg/egg in ovo extract doses. In ovo anise injection increased the number of non-pipped dead embryos. The interaction effect of factors on the hatchability and chick quality were found significant. The results of this study indicate that 9 mg/egg cinnamon, 12 mg/egg ginger, and 3 mg/egg anise extract can be used in in ovo injection without negative effects on the investigated parameters. The role of in ovo cinnamon, ginger or anise extract injection in broiler needs further research.

Keywords: In ovo, Extract, Hatchability, Cinnamon, Ginger, Anise, Chick quality

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1. Introduction

In recent years, the use of medicinal plants and their derivatives in combination with feeding practices has been adopted as an alternative to antibiotics due to the multiple beneficial effects on productivity, immunity, gut development and disease resistance in poultry nutrition (Pathak et al., 2016; Oke et al., 2017; Oke, 2018; Al-Ashoor and Al-Salhie, 2020; Al-Mosawy and Al-Salhie, 2021). A large number of studies have reported that phytobiotics have antimicrobial, anti-inflammatory and transcription modulation potential (Liu, 2004; Kikusato, 2021), have beneficial effects such as inhibiting and reducing pathogenic bacteria (Alcicek et al., 2004; Alshelmani et al., 2021) and can be used as a non-antibiotic growth promoter by virtue of their effects on reducing the inflammatory process, improving gastrointestinal function, increasing growth and production performance and modulating the immune system (Saeed et al., 2020; Kairalla et al., 2022a, 2022b). Among these phytobiotic plants, the effects on broiler growth and physiological responses of cinnamon (Ahmed et al., 2019; Mohammed and Amin, 2019), ginger (Qorbanpour et al., 2018; Daramola et al., 2020; Thomas et al., 2020; Gupta et al., 2021) and anise (Al-Kassie, 2008; Soltan et al., 2008) have been studied and the benefits on performance, immune response, feed digestibility, gut health, meat quality and some blood parameters have been demonstrated. Cinnamon contains various compounds such as sinnamaldehyde, eugenol and carvacrol which have biological activities such as medical treatment, anti-inflammatory, antimicrobial and antioxidant properties (Chang et al., 2013). Ginger contains various compounds and enzymes including gingerdiol, gingerol, gingerdion and shogaols which have antimicrobial, antioxidant and pharmacological effects (Ali et al., 2008; Zhao et al., 2011; Kairalla et al., 2022b). In addition, anise contains various compounds such as sesquiterpene (Wang et al., 2011) anethole, estragole, limonene, linalool and cis-anethole (Dzamic et al., 2009) which have biological activities such as digestive stimulant, antibacterial, antiviral, antifungal, anticancer and antioxidant properties (Mugnaini et al., 2012).

In recent years, in ovo feeding, which offers beneficial biochemical and physiological balances including improved oxidative protection to embryos, has become widespread due to the development of science, technology and breeding in poultry farming and its lower cost (Kadam et al., 2013; Kop-Bozbay et al., 2019; Karamik and Kop-Bozbay, 2020; Atan and Kop-Bozbay, 2021; Kop-Bozbay and Ocak, 2019, 2022). Given that the use of medicinal plants and their derivatives has been proven to have many benefits, in ovo feeding with

bioactive compounds can provide developments for the poultry industry by studying the effects of these compounds on post-hatching immune responses, antioxidant defense and performance as a strategy to improve the health and production performance of poultry. For this purpose, many phytogenic compounds have been tested (Morovat et al., 2016; Zarei et al., 2016; Faseleh Jahromi et al., 2017; Elsaadany, 2019; Ranjbar et al., 2019; Taha et al., 2019; Oladokun and Adewole 2020; Oke et al., 2021; Shehata et al., 2021). However, it is necessary to individually examine each of the numerous phytogenic compounds as alternatives to antibiotics to determine their effectiveness in poultry feeding. Although there are various studies in the literature on the supplementation of cinnamon, ginger or anise to animal rations, there is a lack of information regarding the effect of forementioned extracts at different doses in the same or different studies. For this purpose, in this study aimed, firstly, to evaluate the effects on hatchability and relative weights of yolk, metabolic organ (heart, liver, breast muscle, thigh muscle) and total digestive system (GUT) of in ovo feeding of cinnamon, ginger, or anise extract. Secondly, it was investigated to determine the appropriate dose of cinnamon, ginger or anise extract in in ovo feeding.

2. Material and Method

A total of 480 fertile chicken eggs were collected from a 36-week-old ROSS 308 breeders and were incubated under routine conditions (Çimuka T1280, Ankara, Türkiye).

The eggs were distributed with four replicates of 10 eggs each with an average egg weights as a factorial arrangement of three extracts (cinnamon, ginger, anise) x four doses (0, 3, 9, 12 mg/egg) to hatching trays. On the 18th day of incubation after the fertility control, 1 ml of 0.9% saline solution containing 0, 3, 9, 12 mg of cinnamon, ginger or anise extract was injected into the eggs with a 19 mm and 27-gauge needle. Plant extracts were obtained from a commercial company (Alfasol).

Within two hours of upon hatch all measurements were ascertained. The chicks were weighed and recorded. The hatchability and the number of pipped and non-pipped dead embryos were calculated according to Kop-Bozbay and Ocak (2019). The chick quality (Tona score; Tona et al., 2003) and relative asymmetry (Yalcin et al., 2005) were determined for four randomly selected chicks from each replication. One male and one female chick were selected from each replication to obtain samples from the digestive system and metabolic organs and were euthanized by cervical dislocation. The contents of each chick were opened and the yolk sac, heart, liver, muscles (breast and thigh) and digestive system were carefully removed from the abdominal cavity (Kop-Bozbay and Ocak, 2019) and were weighed and standardized according to live weight.

The collected data was analyzed using the GLM procedure in the SPSS statistical package (SPSS 17.0; SPSS Inc., Chicago, IL, USA). The effect of in ovo plant extract injection, dose and their interaction on all data were analyzed in a randomized block design as a factorial arrangement (3 x 4) of treatments.

3. Result

3.1. Effect of Extract

In ovo feeding with anise extract decreased the hatchability and chick quality compared to other herbal extracts while increased the number of pipped dead embryos ($P<0.05$, Table 1). According to Table 2 in ovo anise extract feeding, increased the relative thigh muscle weight compared to other herbal extracts and decreased the relative proventriculus weight compared to cinnamon extract ($P<0.05$).

3.2. Effect of Extract Dose

Table 1 shows that chick weight was affected by in ovo herbal extract feeding at different doses ($P<0.05$), while hatchability and chick quality were not affected ($P>0.05$). The highest chick weight was found at the 12mg/egg in ovo extract dose ($P<0.05$). The highest relative breast muscle weight was found at the 9mg/egg dose, while the lowest was found at the 0mg/mg dose ($P<0.05$, Table 2). The relative liver and gizzard weights at the 12 mg/egg dose were lower than the other doses and the relative GUT weight was lower than the 0 and 3mg/egg doses ($P<0.05$, Table 2).

3.3. Effect of Interactions

Table 1 shows that extract x dose interaction was observed for hatchability and tona score ($P<0.05$). Increasing the in ovo anise extract dose led to decrease the hatchability and tona score in the anise groups and only tona score in the cinnamon group.

Table 1. The influence of in ovo injection of herbal extracts on the weights of chick (g, CW) and hatchability traits

Extract	Dose	CW	Hatchability of*		EM*		Chick quality**	
			Set eggs	Fertile eggs	Pipped	Non-pipped	Tona score	Relative asymmetry
Cinnamon	Control	40.03	86.39	86.39^{ab}	2.78	10.75	99.50^{a}	1.12
	0.3%	38.93	85.00	89.44^{ab}	5.00	5.00	97.75^{ab}	1.70
	0.9%	39.79	85.00	89.44^{ab}	2.50	7.50	100.00^{a}	1.54
	1.2%	40.77	75.00	92.86^{a}	0.00	5.00	97.38a^{b}	1.03
Ginger	Control	39.92	86.95	88.89^{ab}	0.00	10.50	97.50^{ab}	1.258
	0.3%	39.53	82.50	93.75^{a}	0.00	5.00	98.75^{a}	1.46
	0.9%	39.44	80.00	93.75^{a}	2.50	2.50	99.25^{a}	1.15
	1.2%	40.34	85.00	97.22^{a}	2.50	0.00	99.75^{a}	1.65
Anise	Control	40.078	86.67	86.67^{ab}	2.78	10.50	99.25^{a}	0.95
	0.3%	39.42	85.00	92.22^{a}	7.50	0.00	98.63^{a}	1.20
	0.9%	40.28	62.50	74.11^{bc}	12.50	10.00	86.00^{c}	1.73
	1.2%	41.33	60.00	66.96^{c}	17.50	10.00	92.88^{b}	1.16
Extract								
	Cinnamon	39.88	82.85	89.53^{a}	2.57^{b}	7.06	98.66^{a}	1.35
	Ginger	39.81	83.6	93.40^{a}	1.25^{b}	4.50	98.81^{a}	1.38
	Anise	40.27	73.54	79.99^{b}	10.07^{a}	7.63	94.19^{b}	1.26
Dose								
	Control	40.01^{b}	86.67	87.31	1.85	10.58	98.75	1.11
	0.3%	39.30^{b}	84.17	91.81	4.17	3.33	98.38	1.46
	0.9%	39.84^{b}	75.83	85.77	5.83	6.67	95.08	1.47
	1.2%	40.81^{a}	73.33	85.68	6.67	5.00	96.68	1.28
SEM		0.146	2.209	1.758	1.157	1.003	0.635	0.089
Main effect of	Extract	0.301	0.093	0.002	0.002	0.364	0.001	0.860
	Dose	0.002	0.075	0.411	0.353	0.059	0.062	0.446
	Extract x Dose	0.834	0.341	0.039	0.253	0.354	0.000	0.502

^{a,b,c} Within a row, means with different superscripts differ significantly (P<0.05). EM= embryonic mortality, SEM= standard error of the mean. *The values are means of the four replicates (trays). **The values are means of the eight chicks.

Table 2. The influence of in ovo injection of herbal extracts on relative yolk-sac, metabolically organ, total gastrointestinal tract (GUT) and some digestive system segments weights (g/100 g live weight)

Extract	Dose	Yolk	Breast muscle	Thigh muscle	Heart	Liver	Gizzard	Proventriculus	GUT
Cinnamon	Control	9.66	3.24	11.19	0.77	2.42	4.80	0.90	13.76
	0.3%	9.26	3.36	11.32	0.77	2.29	4.53	0.87	13.46
	0.9%	11.41	6.02	11.57	0.87	2.42	4.54	1.00	13.49
	1.2%	12.20	4.72	11.38	0.75	1.99	3.84	0.84	11.31
Ginger	Control	11.66	3.36	10.65	0.67	2.16	4.59	0.91	12.99
	0.3%	12.68	3.87	11.08	0.77	2.18	4.48	0.80	13.04
	0.9%	11.97	4.31	10.67	0.81	2.39	4.49	0.87	12.68
	1.2%	11.01	3.40	10.79	0.77	1.99	4.31	0.85	12.27
Anise	Control	9.56	3.52	11.53	0.82	2.41	4.57	0.80	13.20
	0.3%	10.26	3.96	12.20	0.83	2.46	4.57	0.85	13.32
	0.9%	13.77	5.18	12.01	0.86	2.42	4.42	0.74	11.53
	1.2%	12.04	4.93	12.34	0.79	2.11	4.02	0.63	10.95
Extract									
	Cinnamon	10.63	4.33	11.37^{b}	0.79	2.28	4.43	0.90^{a}	13.01
	Ginger	11.83	3.73	10.80^{b}	0.76	2.18	4.47	0.86^{ab}	12.75
	Anise	11.41	4.40	12.02^{a}	0.83	2.35	4.39	0.75^{b}	12.25
Dose									
	Control	10.29	3.37^{c}	11.12	0.75	2.33^{a}	4.65^{a}	0.87	13.32^{a}
	0.3%	10.73	3.73^{bc}	11.53	0.79	2.31^{a}	4.53^{a}	0.84	13.27^{a}
	0.9%	12.38	5.17^{a}	11.42	0.85	2.41^{a}	4.48^{a}	0.87	12.57^{ab}
	1.2%	11.75	4.35^{ab}	11.50	0.77	2.03^{b}	4.06^{b}	0.77	11.51^{b}
SEM		0.402	0.187	0.134	0.015	0.049	0.068	0.024	0.209
Main effect of	Extract	0.479	0.191	0.001	0.159	0.356	0.892	0.032	0.256
	Dose	0.261	0.002	0.628	0.111	0.044	0.016	0.384	0.004
	Extract x Dose	0.541	0.384	0.965	0.821	0.967	0.825	0.584	0.542

^{a,b,c}Within a row, means with different superscripts differ significantly (P<0.05). SEM= standard error of the mean. The values are means of the four chicks.

4. Discussion

The embryonic development and post-hatch performance of poultry can be manipulated by applied various nutrients (carbohydrates, amino acids, etc.), phytochemicals, vaccines, and immune system stimulators to eggs using the in ovo technique (Kop-Bozbay et al., 2019; Kop-Bozbay and Ocak, 2019; Hajati et al., 2021; El-Kholy et al., 2021). The application of phytochemicals in in ovo feeding technique has also been shown to have the potential to support these features (Moghaddam et al., 2014; Morovat et al., 2016; Faseleh Jahromi et al., 2017; N'nanle et al., 2017; Khaligh et al., 2018; Al-Shammari et al., 2019; Elsaadany, 2019; Ranjbar et al., 2019; Araujo et al., 2020; El-Kholy et al., 2021; Hajati et al., 2021). These studies demonstrated that phytochemicals can improve antioxidant defense and immunity. However, these effects are influenced by a variety of factors, such as the chemical composition of the extract, extraction method, dose and in ovo injection technique. In the current study, cinnamon and ginger extracts were found to have increased the hatchability by 12-17% compared to the anise group. This result may be attributed to the safrole content of anise. Indeed, the pipped rate was higher than the other groups and the chick quality was lower, which supports this conclusion. Ebrahimnezhad et al. (2011), emphasized that the negative effects of in ovo feeding may be caused by the allergenic properties of the substance used.

In the current study, although the extract factor had no effect in in ovo feeding, the dose (12mg/egg) caused an increase in the hatching weight of the chicks. This increase may be due to the enhanced antioxidant status of the embryos with increasing dose. In addition, plant extracts with high antioxidant content used in our study may have reduced oxidative stress during incubation and thus protected the muscles from oxidative damage (Choi et al., 2016). As a result, this led to an increase in hatchling weight, as well as the relative metabolic organ and GUT weights. Relative thigh muscle weight in the anise group was highest which may be explained by the increase in pipped rate, that is, the survival of strong embryos. When the relative breast muscle, liver and gizzard weights were evaluated together, the most appropriate dose was found to be 3 or 9 mg/egg. This effect may be due to the beneficial effects of phytochemicals on the digestive system, due to their antimicrobial and antioxidant effects (Valenzuela-Grijalva et al., 2017; Yang et al., 2019). When all these physiological changes are evaluated together, it can be said that animals can perform better after hatching.

5. Conclusions

In this study, two important findings were obtained. Firstly, the study has shed light on the usability of cinnamon, ginger or anise extracts in in ovo feeding. Secondly, it has been shown that the use of 9 mg/egg cinnamon, 12 mg/egg ginger and 3 mg/egg anise extract in in ovo feeding can be used without negatively affecting the parameters studied. However, the role of in ovo cinnamon, ginger or anise extract injection in broiler needs further research on the health and performance of chickens.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	C.K.B	B.G.
C	100	
D	100	
S	100	
DCP	50	50
DAI	50	50
L	40	60
W	50	50
CR	50	50
SR	100	
PM	100	
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that all the procedures with animals were approved by the Local Ethics Committee of Animal Experiments of the Eskişehir Osmangazi University (protocol code: HAYDEK-880/2021, date: January 15, 2021).

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