



Effects of dietary turmeric (*Curcuma longa*) on innate and acquired immune responses in broiler chicken

Meysam Khodadadi^a, Nariman Sheikhi^a, Hadi Haghbin Nazarpak^b, Gholamreza Nikbakht Brujeni^{c,*}

^a Department of Clinical Science, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

^b Department of Clinical Sciences, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University, Garmsar, Iran

^c Department of Microbiology and immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

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ABSTRACT

One of the most desired treatments to combat stress and inflammation with minimal adverse effects in large bird populations is food additives. This study investigated the effects of dietary turmeric (*Curcuma longa*) on the level of serum amyloid A (SAA) as an indicator for acute phase response and antibody titer to Newcastle vaccine as an indicator for humoral immune response. A total of 300 Ross broiler chickens were assigned to five dietary groups. Two treatments received basal diets supplemented with different amount of turmeric (250 and 500 mg/kg). One group received aspirin (ASA; 250 mg/L) and one group aspirin-vitamin C (ASA; 250 mg/L + Ascorbic acid; 20 mg/L) in drinking water. There was one control group that received no feeding additives. The levels of SAA and humoral antibody response to Newcastle vaccine were measured during the entire production period. Turmeric administration significantly decreased the serum SAA concentrations after 2 weeks of treatment and later. It also significantly reduced SAA elevation due to the vaccinations on day 17 but not on day 28. After the second vaccination (d 19) ELISA titer in all treatment groups was higher than control group. Significant effect of dietary turmeric on body weight was also found at week 3 and later ages. Administration of 250 mg turmeric per kg diet is recommended for broiler chickens. It is concluded that turmeric is beneficial to minimize inflammatory effects of vaccination in commercial broiler chickens. Turmeric prevents and reduces stress and negative effects of inflammation and stimulates growth performance of broiler chickens.

1. Introduction

Stress in commercial broiler chickens associated with low growth performance, high mortality and poor animal welfare (Scanes, 2016). The main leading cause of stress is thought to be the inflammation due to the infectious and/or noninfectious traumas such as metabolic disorders and neoplasia. Although inflammation, as a nonspecific innate immune response, is essential for defense and repair of damaged tissue, it may have adverse effects on nutrients consumption and production (Broom & Kogut, 2018). Considering the adverse effects of inflammations on immunity and production, implementation of immunomodulatory compounds for commercial breeds seems to be necessary. One of the most desired tools to combat inflammation with minimal adverse effects in large bird populations is food additives.

Varieties of chemicals, such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs), could be used as anti-inflammatory

drugs. Nonetheless, in broiler chickens corticosteroids reduce body and spleen growth and can negatively affect meat production (Post, Rebel & Ter Huurne, 2003). Besides the chemicals, anti-inflammatory effects of some of the natural products and herbal drugs have also been reported (Azab, Nassar & Azab, 2016). Amongst reported products, turmeric (*Curcuma longa*) is well-characterized and successfully used for the management of inflammatory diseases in human (Azab et al., 2016; Bahramsoltani, Soleymani, Rahimi & Farzaei, 2019). To date, anti-inflammatory effects of turmeric has only been reported for fish (Rajabiesterabadi, Hoseini, Fazelan, Hoseinifar & Doan, 2020). In broiler chickens, there are reports that shown the effects of dietary supplementation of turmeric on body performance and humoral immune responses (Ali et al., 2014; Emadi & Kermanshahi, 2006, 2007; Nouzarian, Tabeidian, Toghiani, Ghalamkari & Toghiani, 2011). According to the results obtained in human and species other than birds, it could be suggested that turmeric may act as a potential source of

* Corresponding author at : Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Azadi Avenue, Tehran, Iran
E-mail address: nikbakht@ut.ac.ir (G. Nikbakht Brujeni).

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anti-inflammatory agents for commercial chickens.

Acute phase response (APR) refers to the part of innate immune reactions which play important roles in inflammation, immune modulations, homeostasis and healing. In broiler chickens, APRs are mainly experienced through infection, vaccination, climate changes and nutritional strategies (Janmohammadi, Sheikhi, Nazarpak & Nikbakht Brujeni, 2020; Najafi, Zulkifli, Soleimani & Goh, 2016, 2015; O'Reilly & Eckersall, 2014). Monitoring the APR in chickens endure various kind of stress is of great value for tracking and improving the herd immunity. APR in response to stress can be measured by inflammatory biomarkers such as acute phase proteins (APPs). Alterations in the level of chicken APPs in response to vaccination and mite infestation has been investigated (Janmohammadi et al., 2020; Kaab et al., 2019; Kaab, Bain & Eckersall, 2018). We have previously described the importance of serum amyloid A (SAA) as an indicator for acute phase response in broiler chickens (Janmohammadi et al., 2020). In the current study we measured the effects of dietary turmeric on the levels of SAA and antibody response to Newcastle vaccine during the entire production period. Aspirin (Acetylsalicylic acid; ASA) alone or in combination with vitamin C (Ascorbic acid) were also used in this study for making a comparison between the natural (turmeric) and chemical products.

2. Materials and methods

2.1. Birds housing and vaccination

The study population consisted of 300 one-day-old commercial Ross 308 broiler chicks. All chicks were housed and fed with basic feed according to Ross 308 farming standard protocol (Aviagen, 2018). On day 6, chicks were vaccinated against the Newcastle disease (Nobilis® ND Clone 30, Intervet) by eye drop application. On days 16 and 27, vaccine was given orally through drinking water.

At the end of day 7, chicks were randomly allocated to five dietary groups of equal numbers, 60 per treatment and each treatment was composed of three replications with 20 birds per pen. Two groups received turmeric powder that was added to the basal diet at two different concentrations (250 and 500 mg/kg) for 5 weeks. One group received aspirin (ASA; 250 mg/L) and one group aspirin-vitamin C (ASA; 250 mg/L + Ascorbic acid; 20 mg/L) in drinking water. There was one control group that received no feeding additives. Body weight of birds within each group (10 per replicate) monitored before and after vaccination at days 1, 6, 7, 9, 11, 16, 17, 19, 21, 26, 27, 29, 31 and 36. At the end of study mortality rates for each group was calculated based on the average mortality of pens in each group. Feed intake and body gain were recorded at the end of experiment. The Feed conversion ratio (FCR) was calculated at the end of the experiment as the total feed consumption divided by the weight gain in each treatment group.

2.2. Blood sampling

Blood samples (0.5–1 mL per bird) were collected at 14 sampling time points (3–5 birds per replication within each group) at days 1, 6, 7, 9, 11, 16, 17, 19, 21, 26, 27, 29, 31 and 36. Serum samples were immediately separated and stored at -20°C until analyses. These time points were selected to measure the SAA levels and humoral antibody responses one day before and after each vaccination. The chicks were then kept until the end of their growing period (d 45) and sent to slaughterhouse.

2.3. Assessment of serum amyloid a and humoral antibody response to newcastle vaccine

The enzyme-linked immunosorbent assay (ELISA) kit for chicken (*Gallus*) SAA obtained from Cloud-Clone Corp. (TX, USA). Procedures for measurements were conducted according to manufacturer's instructions. Sandwich (quantitative) ELISA method used for the

measurement of SAA. Serum samples, diluted at 1/5, were added to the ELISA microplate wells coated with anti-SAA antibodies. The calibrator was serially diluted to generate a six-point calibrator curve with a range from 0.003 to 0.2 mg/L concentrations. The wells were incubated for 60 min at room temperature, then washed and biotinylated anti-SAA antibodies was added to the wells and incubated for 1 h at room temperature. Subsequently, HRP-conjugated Streptavidin was added to the wells and incubated for 30 min. The wells were washed and color reaction developed by substrate provided by kit. Finally, the reaction was stopped after 10 min by stop solution and the absorbance of each well determined at 450 nm with a Stat FAX 2000 microtiter plate reader (Awareness Technology, Inc., USA). SAA levels were calculated using the calibration curve.

Humoral antibody response to Newcastle vaccine was measured by ELISA method according to Kaab et al., (2018). Briefly, ELISA plates coated with the Clone 30 and test optimization was conducted using a checker board titration. Accordingly, the optimal concentration used for coating antigen was 7 $\mu\text{g}/\text{ml}$ and the chicken serum samples were diluted 1:100 in PBS-T (PBS containing 0.05% [vol/vol] Tween-20). The coated wells blocked by using PBS-Blotto (PBS containing 0.5% [wt/vol] nonfat dry milk) and incubated at 37°C for 1 hour. After three washing steps, horseradish peroxidase (HRP)-labeled goat anti-chicken IgY (Biorad, Oxford, UK) (1:10,000) was added and incubated for 30 min at 37°C . Then wells washed an additional three times and a color reaction developed by using tetramethyl benzidine (TMB) (Sigma Aldrich, St Louis, MO, USA) as substrate. Finally, the reaction was stopped after 10 min by H_2SO_4 (3 M) and the absorbance of each well determined at 450 nm with a Stat FAX 2000 microtiter plate reader (Awareness Technology, Inc., USA).

2.4. Statistical analysis

Significant differences between groups were analyzed by the unpaired two-tailed Student's *t*-test. For multiple mean comparisons, analysis of variance (ANOVA) and post-hoc tests was used. Repeated-measures ANOVA was used to test for significant differences in different sampling time points. Statistical significance is considered as $P \leq 0.05$.

3. Results

At 17 days of age, chickens fed aspirin-vitamin C or 500 mg/kg turmeric showed significantly ($P \leq 0.05$) higher mean body weight than control group (Table 1). At d 26, chickens fed aspirin or aspirin-vitamin C showed lower ($P \leq 0.05$) mean body weight compared to control and both turmeric treated (250 or 500 mg/kg) groups. At d 29, only chickens fed 250 mg/kg turmeric showed higher mean body weight ($P \leq 0.05$) compared to control group. At d 31, chickens fed 250 mg/kg turmeric showed significantly higher mean body weight compared to chickens fed aspirin-vitamin C. At d 36, those chickens that fed 500 mg/kg turmeric showed significantly higher mean body weight compared to all treated groups. Furthermore, chickens fed aspirin-vitamin C showed higher ($P \leq 0.05$) mean body weight compared to control group. At the end of the experimental period (36 days of age), 500 mg/kg turmeric treated group showed the lowest FCR (Table 2). No significant differences were found between aspirin, aspirin vitamin C and 250 mg/kg turmeric treated groups.

At 17 days of age, chickens fed 500 mg/kg turmeric showed significantly ($P \leq 0.05$) lower ELISA titer than control group (Table 3). At d 19, all treated groups showed higher ($P \leq 0.05$) titers when compared to control group. At the same time chickens fed aspirin-vitamin C showed higher titer than all treated groups. At d 29, chickens in control group showed higher titer than all treated groups. At d 36, both chickens in control and aspirin-vitamin C groups showed higher humoral antibody response compared to all other treated groups.

The serum level of SAA at each sampling time point of experiment

Table 1

Body weight of commercial broiler chickens reared in five dietary groups during 36 days of experiment. Data presented as mean and standard deviation.

Experimental group* Age (days)	Aspirin		Aspirin-Vitamin C		Turmeric 250		Turmeric 500		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	38.9	0.35	38.57	1.28	38.43	1.15	37.77	2.31	38.9	0.72
6	98.85	1	98.56	0.49	98.52	0.58	99.18	0.58	98.18	1.15
7	117.21	1.15	116.54	4	114.87	1.53	117.87	2.31	114.87	3.79
9	151.67	20.01	150.33	10.96	152.33	9.07	143	18.73	137.33	15.31
11	190.33	23.35	185	25.71	187.33	38.08	205.67	23.86	194	38.11
16	270.67	46.92	314	37.03	284.33	68.09	280	46.78	316.33	18.77
17	324 ^{A,C}	49	365.67 ^{A,B}	62.32	342	34.39	397.67 ^B	36.09	291.33 ^C	66.30
19	465	61.88	456.33	16.04	471.33	54.45	463.33	35.25	461.33	84.18
21	533	34.22	507.67	19.65	511.67	104.52	558.33 ^A	46.19	482.33 ^B	41.58
26	861.33 ^A	122.41	813 ^A	124.72	971 ^B	23.06	943.67 ^B	11.72	947 ^B	58.41
27	1082.33 ^A	124.48	1018.67 ^A	157.75	1064.33 ^A	94.68	1037.67 ^A	51.93	1180.67 ^B	75.26
29	1161	59.25	1160.33	106.87	1222.33 ^A	65.58	1124.67	115.69	1069 ^B	98.22
31	1295.33	130.86	1207.67 ^A	183.83	1343.33 ^B	214.18	1258	79.89	1277.67	206.32
36	1730 ^{A,C}	132.54	1835 ^A	100.68	1742.5 ^{A,C}	95.03	2050 ^B	85.54	1686.5 ^C	100.38

* Values having a common uppercase letter are not significantly different ($P \leq 0.05$).**Table 2**

Body weight (g/bird), weight gain (g/bird), feed consumption (g/bird), and feed conversion ratio (FCR) of broilers from beginning of the treatment (7d) to end of the experiment (36d). Data presented as mean and standard deviation.

Group	BW 7d	BW 36d	Feed consumption	BW gain	FCR	Mortality rate (%)
Aspirin	117.21 ^A ± 1.15	1730 ^{A,C} ± 132.54	2849.72 ^{A,C} ± 192.51	1646.46 ^A ± 86.95	1.73 ^{A,C} ± 0.04	10.1 ^A ± 7.1
Asp-Vit C	116.54 ^A ± 4.00	1835 ^A ± 100.68	2886.11 ^A ± 150.89	1718.69 ^A ± 102.40	1.68 ^A ± 0.04	15.0 ^A ± 7.1
Tur- 250	114.87 ^A ± 1.53	1742.5 ^{A,C} ± 95.03	2653.03 ^{A,C} ± 273.12	1626.96 ^{A,C} ± 153.44	1.63 ^{A,C} ± 0.07	3.33 ^{B,C} ± 2.3
Tur- 500	117.87 ^A ± 2.31	2050 ^B ± 85.54	3001.59 ^B ± 148.94	1936.12 ^B ± 70.21	1.51 ^B ± 0.01	0.0 ^C ± 0.0
Control	114.87 ^A ± 3.79	1686.5 ^C ± 100.38	2801.32 ^C ± 74.73	1569.46 ^C ± 88.08	1.78 ^C ± 0.01	10.3 ^A ± 4.1

^{a-c} Means with similar superscripts alphabet in the same column did not differ significantly ($P < 0.05$).**Table 3**

ELISA absorbance values of commercial broiler chickens reared in five dietary groups during 36 days of experiment. Chickens vaccinated against Newcastle disease at days 6, 16 and 27. Data presented as mean and standard deviation.

Experimental group* Age (days)	Aspirin		Aspirin-Vitamin C		Turmeric 250		Turmeric 500		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.877	0.34	0.895	0.28	0.784	0.30	0.903	0.24	0.869	0.15
6	0.549	0.02	0.516	0.01	0.540	0.06	0.618	0.08	0.561	0.03
7	0.555	0.04	0.537	0.05	0.575	0.03	0.574	0.09	0.557	0.04
9	0.549	0.05	0.547	0.09	0.599	0.03	0.522	0.05	0.559	0.06
11	0.546	0.07	0.584	0.10	0.551	0.05	0.510	0.05	0.494	0.06
16	0.501	0.06	0.500	0.02	0.527	0.08	0.517	0.13	0.491	0.02
17	0.542	0.05	0.572 ^A	0.05	0.458	0.06	0.434 ^B	0.01	0.572 ^A	0.09
19	0.787 ^A	0.28	0.973 ^B	0.78	0.807 ^A	0.09	0.712 ^A	0.12	0.515 ^C	0.08
21	0.619 ^A	0.24	0.503	0.11	0.561	0.11	0.439 ^B	0.01	0.503	0.07
26	0.465	0.02	0.477	0.04	0.501	0.07	0.530	0.10	0.534	0.15
27	0.488	0.04	0.550	0.09	0.545	0.04	0.439	0.04	0.548	0.09
29	0.528 ^A	0.04	0.555 ^A	0.11	0.456 ^A	0.04	0.541 ^A	0.09	0.728 ^B	0.25
31	0.486	0.03	0.593 ^A	0.15	0.449 ^B	0.09	0.533	0.07	0.551	0.09
36	0.618 ^A	0.08	0.868 ^{B,C}	0.12	0.590 ^A	0.13	0.560 ^A	0.05	0.912 ^C	0.02

* Values having a common uppercase letter are not significantly different ($P \leq 0.05$).

was illustrated in Fig. 1 (Fig. 1). In all groups SAA rapidly increased after first vaccination (d 6) and continued to rise for 2 days (d 9). Treatment was started at day 7 but no significant change was observed before second vaccination. A highly significant increase in SAA level was also observed at days 19 and 21 in control group (2 days post vaccination). The same increment was noted for groups treated with turmeric 500 mg/kg and aspirin. Interestingly, only in the group fed with 250 mg/kg turmeric, SAA significantly decreased at weeks 2 and 3 after treatment (d 21–36).

In comparisons between treatments and control group, after second vaccination (d 17), SAA level in treatment groups of turmeric 250 and aspirin-vitamin C was significantly lower than control group (Fig. 1). Before and after third vaccination (d 27) no significant difference was

found between control and turmeric treated groups. However, before third vaccination, SAA in aspirin and aspirin- vitamin C treatment groups was significantly higher than the control group (d 27). At day 36, SAA level in treatment group of turmeric 250 was also lower than control group.

Table 4 shows significant differences found between SAA levels of treatment groups. Four days after the beginning of treatment (d 11), serum SAA concentration in group fed with aspirin was significantly lower than group fed with 250 mg/kg turmeric. The same effect of aspirin on SAA level was also observed at day 19. Again at the same day, group fed with 500 mg/kg turmeric showed a lower SAA level when compared to group fed with lower concentration of turmeric (250 mg/kg). A highly significant decrease in SAA levels was found at day 36 for

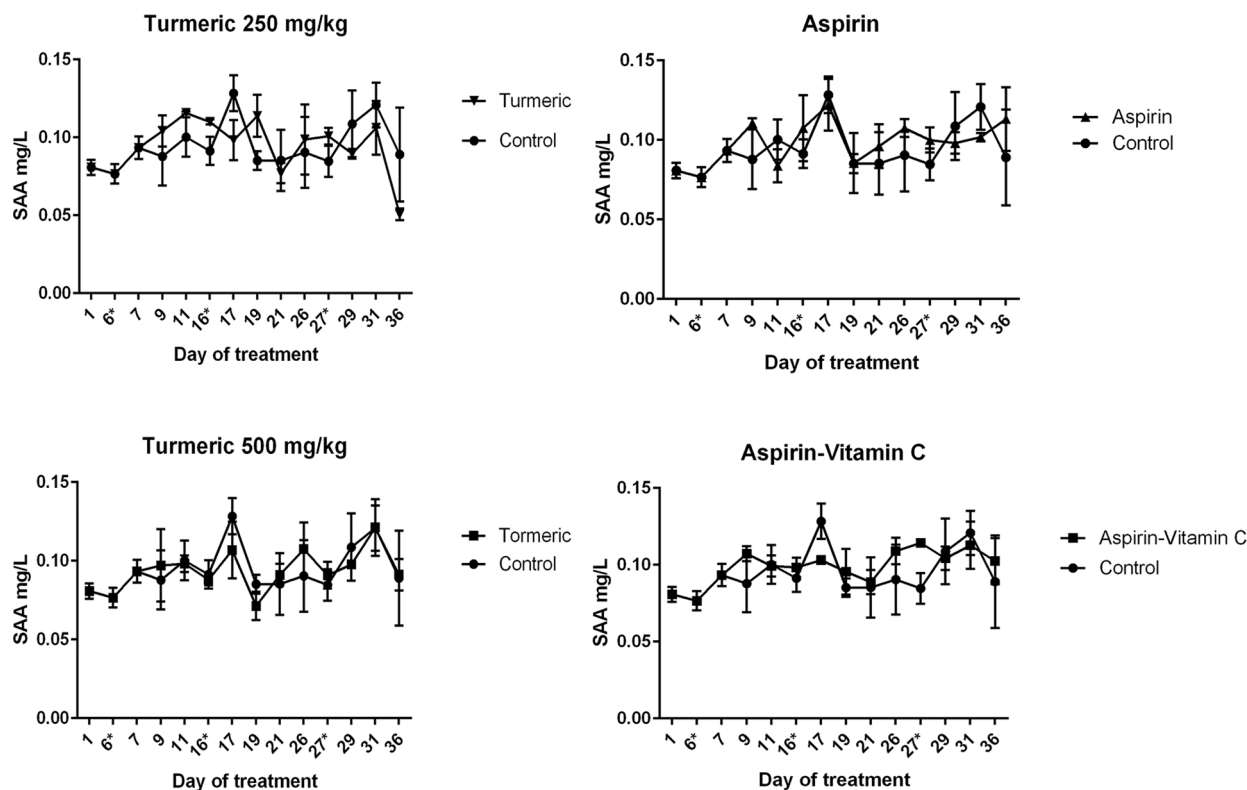


Fig. 1. Serum SAA concentrations during feeding with turmeric or aspirin supplemented diets. Chickens were left untreated (Control), or were treated with turmeric 250 mg/kg, turmeric 500 mg/kg, aspirin or aspirin-Vitamin C. *Chickens vaccinated against Newcastle disease at days 6, 16 and 27.

Table 4
Significant differences between SAA levels in chicken treated with turmeric (250 or 500 mg/kg) or aspirin (aspirin or aspirin-vitamin C) supplemented diets.

Day	Treatment groups	SAA Mean Differences (mg/L)	P-Value
11	Turmeric 250 vs. Aspirin	0.032	0.0155
19	Turmeric 250 vs. Aspirin	0.028	0.0318
	Turmeric 500 vs. Turmeric 250	-0.042	0.0014
36	Turmeric 250 vs. Aspirin	-0.063	<
	Turmeric 250 vs. Aspirin-Vitamin C	-0.052	0.0001
	Turmeric 500 vs. Turmeric 250	0.041	0.0022

group treated with 250 mg/kg turmeric compared to groups fed with aspirin, aspirin-vitamin C as well as 500 mg/kg turmeric (Table 1). No significant change in SAA level was detected in any other groups during the month of treatment. Mean SAA value during the experimental period was compared between treatment groups. Chicken feeding with 250 mg/kg turmeric led to decreases in serum SAA compared to other treatments.

Mortality of the 300 birds during the 6 weeks of experimental period was 23 (7.6%). Mortality was markedly higher in aspirin-vitamin C treated broilers (15%) and lower in group fed 500 mg/kg turmeric (0%). The mortality rate in control group, aspirin and 250 mg/kg turmeric was 10, 10 and 3.33%, respectively.

4. Discussion

It is known that management and vaccinations in commercial chickens results in stress due to inflammation and elevated acute phase proteins (Janmohammadi et al., 2020; Kaab et al., 2018). Inflammation can also make chickens more prone to infectious and metabolic disease. In the present study, SAA as a marker of inflammation and an indicator

of acute phase response was reduced by using the diet supplemented with 250 mg per kg turmeric. To the best of our knowledge, there is no study reporting effects of turmeric on acute phase response in commercial broiler chicken. Dietary effects of rhizome powder of turmeric on performance, carcass characteristics and blood enzymes of broiler chickens have been investigated (Emadi & Kermanshahi, 2006, 2007). The results showed that adding 500 or 750 mg/kg turmeric powder to diet can improve carcass quality. In our experiment significant effect of dietary turmeric on body weight was found at week 3 and later ages. Orally administered turmeric requires a longer-term regimen due to low intestinal absorption, rapid metabolism and elimination from the systemic circulation. However, in the case of desired biological activities, dose and duration of orally administered turmeric varied from a week to more than 3 months as shown in vivo studies (Gupta et al., 2013).

A markedly higher mean body weight was observed at week 6 for chickens that fed 500 mg/kg turmeric (Table 1). The differences between the levels of humoral antibody response against ND vaccine amongst studied groups were also found at week 3 and later (Table 3). It should be noted that only after second vaccination (d 19) raising titers in all treatment groups was higher than control group. Reduced levels of humoral antibody response in broilers fed aspirin, vitamin C and turmeric might be due to the immunomodulatory or anti-inflammatory effects. In a normal situation or status quo, even if the humoral antibody response decreases, but still remains at the protective level, it might be useful for growth performance. Turmeric can reduce negative effects of stress and inflammation on growth performance of broiler chickens. Amongst over 300 different components of dietary turmeric, only Curcuminoids has been extensively investigated. Curcuminoids are a mixture of curcumin, desmethoxycurcumin and bisdemethoxycurcumin. These major polyphenolic compounds of turmeric (3–6%) are responsible for various biological activities (Niranjan & Prakash, 2008). It has been demonstrated that turmeric has different immunomodulatory effects. It exhibited stimulatory effects on the proliferation of human peripheral blood mononuclear cells (Gupta et al., 2013). In rats 5 weeks dietary of

curcumin could significantly enhance the IgG levels. In vitro and in vivo anti-inflammatory and antioxidant activities of curcumin have been also investigated (Niranjan & Prakash, 2008).

Routine vaccination programs induce acute immune response in both commercial layer and broiler chickens (Janmohammadi et al., 2020; Kaab et al., 2018). In the current study SAA level normally increased one day after each vaccination on days 16 and 27 (Fig. 1). Turmeric at the concentration of 250 mg/kg and aspirin-vitamin C significantly reduced SAA elevation in broilers due to the vaccination on day 17 but this effect was not significant on day 28. Anti-inflammatory effects of turmeric has been reported for human and fish (Rajabiesterabadi et al., 2020; White, Pasupuleti, Roman, Li & Hernandez, 2019). In both studies cytokines related to inflammation were measured. Inflammatory cytokines involved in triggering and developing the acute-phase response. Somehow the anti-inflammatory cytokines can effectively terminate acute-phase responses (Koj, 1998). In chicken the effects of turmeric on inflammatory cytokines as well as the correlation between these cytokines and the acute phase response remains to be elucidated.

We could not show any significant effects of solo aspirin on SAA in the studied population. To date only the protective functions of aspirin against heat stress has been reported (Wu et al., 2016). It has been also demonstrated that aspirin in combination with vitamin C can be used for preventing the heat stress and improving the performance of broiler chickens (Roussan et al., 2008). However, the supplementary effect of vitamin C on aspirin needs more investigations.

The decrease in serum SAA level was found to be around two weeks after treatment (d 19). Aspirin treated group showed more decrease on days 11 and 19 compared to the turmeric treatment group (Table 1). At the same day chickens treated with 250 mg/kg turmeric had lower SAA compared to group treated with 500 mg/kg. At the end of experiment (d 36) the reducing effects of 250 mg/kg turmeric on SAA level was significantly higher when compared to all other treatment groups (Table 1). Both aspirin and turmeric are commonly used to treat human inflammatory disorders but there is no studies reporting comparison between these two chemical and herbal medicines in human and animal species (Mohapatra, Sarkar, Dash, Moharana & Subudhi, 2018).

5. Conclusion

It is concluded that turmeric administration is beneficial to minimize inflammatory effects of vaccination and help growth performance in commercial broiler chickens. Turmeric is a potential source of anti-inflammatory agents. Administration of 250 mg per kg dietary turmeric is recommended to reduce acute phase response due to the vaccination and can stimulate growth performance in broiler chickens.

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Data availability

The data that support the findings of this study are included in the manuscript.

Ethical statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The study was approved by Institutional Ethical Committee of Islamic Azad University, Tehran, Iran. The animals were handled according to high ethical standards and national legislation.

CRediT authorship contribution statement

Meysam Khodadadi: Conceptualization, Data curation, Writing – original draft. **Nariman Sheikh:** Conceptualization, Formal analysis, Writing – original draft. **Hadi Haghbin Nazarpak:** Data curation, Formal analysis. **Gholamreza Nikbakht Brujeni:** Conceptualization, Formal analysis, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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