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Behavior, Fear Response, Performance and Immune Response of Broiler Chicks Fed Graded Levels of Anise Seed as Alternative to Antibiotics

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ABSTRACT

A total of two hundred twenty five day-old Cobb broiler chicks were distributed into five groups (15 birds/ group, three replicates for each treatment). The dietary treatments were designed to fed the chicks of basal diet without supplementation (control), basal diet supplemented with 0.5, 0.75 and 1gm/kg anise seed, and basal diet supplemented with 10g/kg zinc bacitracin (anti-biotic group). Behavioral observation was carried out twice daily (7:00-11:30 am and 13:00-17:30 pm) two days a week for 6 consecutive weeks. Body weight (BW) and feed intake per pen were measured weekly from which body weight gain (BWG) and feed conversion ratio (FCR) were measured. Immune response against NDV was determined at 33th day of age. At the end of experiment, fear responses, differential leucocyte count, Hb concentration, PCV, and some carcass traits were assayed. Dead birds from each treatment were recorded. The obtained results showed that higher proportion of birds supplemented with anise 0.5, 0.75 and 1.0 gm/kg were engaged in more feeding behavior compared to control one. Birds supplemented with anise seed and antibiotics were engaged in significantly more resting behavior compared to birds in control group. Untreated control birds exhibited significantly higher preening behavior and lower wing stretching and/or wing flapping and dust bathing behaviors compared to anise seed fed groups. At the 43th day of age birds supplemented with 0.5, 0.75 and 1.0 gm/kg anise seed had significantly higher BW, BWG, and better FCR and had higher carcass percentage compared to birds in control group, but not significantly differed from birds in antibiotic group. Mortality rate was not differed among different treatments. Percent of live weight of bursa and Hb content were significantly higher in anise seed supplemented groups compared to control and anti-biotic groups. Chicks fed basal unsupplemented diet were more fearful in tonnic immobility test and were more stressed and had a significant increase in heterophil / lymphocyte ratio when compared with those in all supplemented diet groups. In contrast, dietary supplementation had no significant effect on antibody titer against NDV. In conclusion, dietary supplementation of broiler chicks with anise 0.5, 0.75 and 1.0 gm/kg improved feeding and comfort behaviors, improved productive performance and bird's welfare (reduced fear and stress) with no effect on immune response. Anise seed could be used as natural alternatives to feed antibiotics in broilers feed.

1. INTRODUCTION

Sub-therapeutic dosage of antibiotics has been used as growth promoters in the livestock diets to improve growth performance, feed efficiency and protect bird's health (Collington et al., 1990). Ferket (2004) reported that antibiotic as fed additives decreased the total microbial load in the gut which resulted in reduced metabolic energy output intended for gut

maintenance, this extra energy is used for performance and lead to better growth of supplemented birds (Hashemi and Davoodi, 2011). The concern of antibiotic-resistant pathogens and antibiotic residues resulted in ban of these products by the European Union in January 2006 and has encouraged the search for alternatives growth promoters such as probiotics, prebiotics, enzymes, organic acids and phytogenic additives (Demir et al., 2003; Hernandez et al., 2004; Sarica et al., 2005).
The plant origin products are appropriate and better feed additives because they are natural, residue free and less toxic (Wang et al., 1998). Phytochemicals could be divided into four groups includes, Herbs, Botanicals, Essential oils and Oleoresins (Windisch & Kroismayr, 2006). It have antimicrobial and manipulate gut microorganisms (Cowan, 1999; Hashemi and Davoodi 2011), immune stimulant (Guo et al., 2004) anti-coccidial (Allen et al., 1997; Youn and Noh, 2001), antioxidants (Lopez-Bote et al., 1998; Botsoglou et al., 2002), hypocholesterolemics (Craig, 1999), manipulating the gut function (Collington et al., 1990) and improved liver function (Hernandez et al., 2004).

Anise (Piminella anisum L.) is a yearly aromatic plant intuitive to Iran, India, Turkey and Egypt and several other warm areas in the world. As a medical herb, anise has been used as digestive stimulant and anthelmintic (Cabuk et al., 2003), antimicrobial (Singh et al., 2002), expectorant, carminative and antispasmodic (Zepernicket al., 1984), antioxidants (Gulcin et al., 2003) hypocholesterolemic (Craig, 1999), anticonvulsant (Pourgholam et al ., 1999), anti-fungal (Soliman and Badea, 2002), antipyretic (Afifi et al., 1994) and antiepileptic (Janahmadi et al ., 2008), increased milk production in lactating women and relief gastrointestinal troubles from her nursing infants (Zargari, 1996), muscle relaxant effect (Albuquerque et al.,1995) and laxative (Chicouri and Chicouri, 2000). Anise seed is registered by the European Council as natural source of feed flavor and in the USA as GRAS, i.e. Generally recognized as safe (Franz et al., 2005; Al-Beitawi et al., 2009).
However, several studies have been conducted to determine the effect of dietary supplementation of anise on the performance of broiler chicks (Ciftci et al., 2005; Soltan et al., 2008; Al-Beitawi et al., 2009 and 2010; Yazdi et al., 2014; Mahmood et al., 2014; Alhajj et al. 2015), no available study describes their behavioral and welfare effects have been formerly reported. Anise seeds as additives for laying Japanese quail resulted in significant changes in the color of the egg yolk and lowering the cholesterol level in the serum, with no effect on bird's performance and egg qualities (Christaki et al., 2011), and improved immune response to NDV (Bayram et al. 2007).
The current study was designed to investigate the effects of graded levels of anise seed as alternative to antibiotic on the behavior, welfare, performance, some carcass traits, some blood parameters and immune response against New castle Diseased Virus of broiler chicks.

2. MATERIALS AND METHODS

2.1. Experimental birds

Two hundred twenty five day-old Cobb broiler chicks were obtained from commercial hatchery. Chicks were weighed on arrival and randomly distributed into five dietary treatments. Each treatment was housed in floor pen (15 birds per pen), with three replicates for each treatment (45 birds for each treatment). The light regimen in the house was 23 hr. Temperature was reduced from 34 ^{o}c at first day to 25 ^{o}c at three weeks and was then kept constant. Food and water were provided *ad libitum*. Birds fed on commercial diet free from antibiotics (table, 1), International Company for Feed and Animal Production (IFAP). The dietary treatments were consists of basal diet (control), basal diet supplemented with 0.5gm/kg anise seed, basal diet supplemented with 0.75gm/kg anise seed, basal diet supplemented with 1.0gm/kg anise seed and the antibiotic group received basal diet supplemented with 10g/kg zinc bacitracin. Dietary treatment started from day one to day 43 of age (end of the experiment). A standard vaccination program was applied during the whole period for all birds.

Table 1. Composition of basal ration.

Ingredient	
Crude protein %	22.06
Digestable energy Kcal/ kg of diet	3079
Calcium %	1.2
Phosphorus %	0.44
Lysine %	0.75
Methionine and cystine %	0.82

2.2. Measurements

2.2.1. Behavioral observations

Chicks of each treatment were directly observed in two periods/ day, one period was in the morning (7.00-11.30) and the 2^{ed} was in the afternoon (13.00-17.30). Each pen was observed for 15 minutes in each observation period. Bird's observation was performed two days/ week for six consecutive weeks. Instantaneous scan sampling observations of chick's behavior were performed (Lee and Craig, 1990). The number of chicks engaged in ingestive behavior (feeding and drinking) and comfort behaviors [resting (sitting, lying or sleeping- with no other behavior), preening, wing related behaviors as wing/leg stretching and/or wing flapping, and dust bathing] was recorded. The percentage of chicks engaged in each behavior was calculated during all scan samples.

2.2.2. Performance

Birds of each pen were individually weighed weekly till the end of the experiment (BW), weekly feed intake (FI) per pen was measured. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated for each dietary treatment. At forty three days of age, three birds per pen were randomly chosen, weighed, slaughtered and their spleen, heart, liver, bursa and gizzard were weighed and calculated as a percentage of live body weight. Mortality rate was recorded for each pen.

2.2.3. Hematological parameters.

At the 43th day of age, blood samples were collected (four chicks from each pen, 12 chicks per treatment). The blood was received into heparinized tubes for the determination of hemoglobin content (Hb) and packed cell volume (PCV) (Wintrobe, 1976). Blood smears were prepared and stained with May-Grunewald-Giemsa stain. One hundred leucocytes, including heterophils, eosinophil, lymphocytes, and monocytes were counted at ×40 (oil immersion lens) and heterophil to lymphocyte ratio (H/L) was calculated.

2.2.4. Tonnic immobility (fear test).

Tonic immobility (TI) reaction was assessed on five birds per replicate for 5–min test period, as described by Jones and Faure (1981) and Mills and Faure (1991)

2.3. Statistical analysis

Differences between groups were analyzed using ANOVA test. Statistical analyses were performed using the General Linear Models (GLMs) procedure in SAS, 2001, version 8. All data are expressed as the LS mean ± SE.

3. RESULTS AND DISCUSSION

3.1 Ingestive and comfort behaviors

The effects of dietary supplementation of different levels of anise seed and antibiotics on the ingestive and comfort behaviors of broilers are presented in table 2. Higher proportion of birds supplemented with anise 0.5, 0.75 and 1.0 gm/kg diet were recorded as engaged in more feeding behavior ($p<0.05$) compared to control one, with birds supplemented with antibiotics were intermediary but not different from them ($p>0.05$). This result was in close agreement with Al kassi (2008) who found that broiler chicks fed .5% and 1% anise seed consumed more food than control birds. Also, Al-Beitawi et al. (2009) reported that anise seeds as alternative to antibiotics growth promoter significantly improved feed intake compared to birds fed basal diet. Moereover, Hamodi and Al-Khilani (2012) indicated that birds fed 0.4% and 0.6% anise seed consumed more feed than birds feed control diet.

The increased feed intake in anise supplemented groups may be due to appetizing effects of ingredients such as anethole (cabuk et al., 2003).

Contrarily to results of the current study, supplementation of diet with graded levels of anise seed (0.25% to 1.5%) had no effect on broilers feed consumption (Soltan et al. 2008). Also, Yazdi et al. (2014) found decreased average daily feed intake of Ross broilers fed 1%, 5% or 10% anise seed supplemented diets compared to control birds however the differences not reach to significant value. Ciftci et al. (2005) reported that anise oil 100mg/kg, 200 mg/kg, 400 mg/kg or antibiotic supplementation to broilers diet had no effect on daily feed intake. Also, El-Deek et al. (2002) indicated that anise seed, ginger or fennel either separately or as a mixture had no effect on feed intake. In addition, Alhajj et al. (2015) revealed that, Chinese Star Anise 1, 2, 4, 6 or 8 gm/kg diet had no effect on feed intake.

Table 2. Means and their standard errors of the effect of dietary supplementation with anise seed and antibiotic on ingestive and comfort behaviors of broiler chicks.

	Control	0.5gm anise	0.75gm anise	1.0gm anise	Antibiotic	p-value
Ingestive behavior						
Feeding	10.06^{b}±1.07	14.16^{a}±1.22	14.40^{a}±1.39	14.76^{a}±1.37	12.26^{ab}±1.19	0.049
Drinking	5.69±.87	5.35±0.82	4.28±.64	3.80±0.67	4.04±.84	Ns
Comfort behavior						
Resting	45.45^{b}±2.47	53.33^{a}±1.86	56.30^{a}±2.03	58.69^{a}±1.71	55.47^{a}±2.00	0.00
Preening	10.42^{a}±1.25	6.30^{b}±0.85	6.07^{b}±.76	6.54^{b}±0.96	8.69^{ab}±1.00	0.006
wing/leg stretching and/or wing flapping	1.45^{b}±0.37	3.45^{a}±0.61	4.28^{a}±.72	3.80^{a}±0.65	2.73^{ab}±.47	0.008
Dust bathing	$.12^{b}$±0.12	1.19^{a}±0.41	1.30^{a}±0.43	1.42^{a}±.47	$.47^{ab}$±.23	0.043

Means within the same row carry different superscripts are significantly different.

Table 3. Means and their standard errors of the effect of dietary supplementation with anise seed and antibiotic on productive performance of broiler chicks

	Control	0.5gm anise	0.75gm anise	1.0gm anise	Antibiotic	p-value
Initial weight	43.52±1.00	43.51±1.05	43.50±0.92	42.00±1.12	42.62±1.05	ns
Final weight	1807.80b±47.54	1985.00a±25.05	1984.26a±35.83	1990.40a±33.58	1879.80ab±35.66	0.001
Weight gain	1764.27b±47.98	1941.48a±25.30	1940.76a±36.32	1948.39a±33.45	1837.18ab±36.12	0.001
FCR	1.88a±0.05	1.71b±0.02	1.71b±.03	1.72b±0.02	1.80ab±.03	0.002

Means within the same row carry different superscripts are significantly different.

Table 4. Means and their standard errors of the effect of dietary supplementation with anise seed and antibiotic on carcasses traits of broiler chickens (43 days of age).

	Control	0.5gm anise	0.75gm anise	1.0gm anise	Antibiotic	p-value
Carcass %	0.74^{b}±0.01	$.78^{a}$±0.009	0.80^{a}±0.010	0.78^{a}±0.012	0.77^{ab}±0.01	0.013
Spleen	0.12±0.01	0.15±0.01	0.14±0.01	0.15±0.03	0.15±0.01	Ns
Gizzard	1.33±0.06	1.44±0.08	1.39±0.06	1.49±0.20	1.62±0.09	Ns
Liver	2.60±0.24	2.53±0.26	2.64±0.30	2.81±0.43	2.97±0.48	Ns
Heart	0.50±0.04	0.51±0.03	0.49±0.05	0.59±0.04	0.65±0.10	Ns
Bursa	0.05^{b}±0.002	0.10^{a}±0.006	0.10^{a}±0.011	0.12^{a}±0.015	0.06^{b}±0.011	0.002
Mortality rate	4.44	2.22	0.00	0.00	2.22	Ns

Means within the same row carry different superscripts are significantly different.

Table 5. Means and their standard errors of the effect of dietary supplementation with anise seed and antibiotic on differential leucocyte count Hb content and PCV of broiler chicks (42 days of age)

	Control	0.5gm anise	0.75gm anise	1.0gm anise	Antibiotic	p-value
Heterophil	13.25^{a}±0.94	8.00^{b}±0.70	6.25^{b}±0.62	7.75^{b}±0.47	8.00^{b}±1.0	0.001
Lymphocyte	80.50^{c}±1.50	87.00^{ab}±0.91	88.75^{a}±0.47	86.75^{ab}±0.62	84.00^{b}±1.08	0.001
Monocyte	3.00±0.40	2.75±0.75	0.50±0.28	2.00±0.40	2.75±0.47	Ns
Eosinophil	3.00±1.08	1.50±0.50	2.25±0.94	2.00±0.40	2.75±0.47	Ns
Basophil	1.25±0.62	0.75±0.75	0.50±0.28	0.50±0.28	1.00±0.40	Ns
H/L Ratio	0.181^{a}±0.01	0.085^{b}±0.01	0.070^{b}±0.01	0.088^{b}±0.01	$.088^{b}$±0.01	0.001
Hb	0.46^{b}±0.039	0.62^{a}±0.027	0.62^{a}±0.022	$.63^{a}$±0.030	0.49^{b}±0.023	0.001
PCV	22.83±3.85	26.33±4.92	28.50±2.37	29.50±2.84	27.83±0.83	Ns

Means within the same row carry different superscripts are significantly different.

Table 6. Means and their standard errors of the effect of dietary supplementation with anise seed and antibiotic on fear response (tonnic immobility duration, TI).

	Control	0.5gm anise	0.75gm anise	1.0gm anise	Antibiotic	p-value
TI (sec.)	218.67a±22.59	97.78b±26.76	125.22b±28.45	109.56b±34.67	100.11b±0.024	0.024

Means within the same row carry different superscripts are significantly different.

Table 7. Means and their standard errors of the effect of dietary supplementation with anise seed and antibiotic on antibody titer (log2 HI) against NDV.

	Control	0.5gm anise	0.75gm anise	1.0gm anise	Antibiotic	p-value
Antibody titer	3.20±0.37	3.40±0.67	3.80±0.58	4.00±0.71	3.20±0.48	Ns

Means within the same row carry different superscripts are significantly different.

The comfort behavior patterns (Table 2) revealed significant differences in different comfort behavior measured in the current study. Birds supplemented with graded levels of anise seed and antibiotics were engaged in significantly more resting behavior compared to birds in control group. Wing stretching and/or wing flapping and dust bathing behaviors were significantly higher in birds supplemented with anise seed compared to control birds, while in the antibiotic group there were no differences compared to control or anise seed supplemented groups. Control birds exhibited significantly higher preening behavior compared to anise supplemented groups, with birds supplemented with antibiotics were intermediary but not different from them. The increased comfort behavior in the anise supplemented groups may related to the chemical ingredients of anise seed which enhance immunesystem of the bird and reduce the effect of stress and consequently reflected on bird's comfort behavior and welfare.

3.2. Productive performance:

In the current study, body weights (BW), body weight gain (BWG) and feed conversion ratio (FCR) were significantly influenced by the addition of anise seed and antibiotic to diets (Table 3). At the 43th day of age birds supplemented with 0.5, 0.75 and 1.0 gm/kg anise seed had significantly higher BW, BWG and better FCR compared to birds in control group, but not significantly differed from birds in antibiotic group. Similarly, Al–Kassie (2008) reported that daily live BWG was significantly higher and FCR was superior in birds fed 1% and 0.5% anise seed compared to control birds. Al-Beitawi et al. (2009) reported that anise seeds as alternative to antibiotics significantly improved BW, BWG and FCR compared to birds fed basal diet. Soltan et al. (2008) reported that broilers fed 0.5 and 0.75 gm/kg of anise seed had significantly improved final BW, performance index and BWG compared to control birds while, .25, 1.0 and 1.25 gm resulted in non-significantly improvement, although at 1. 5 gm bird's performance was significantly reduced. Hamodi and Al-Khilani (2012) revealed that birds fed 0.4% and 0.6% anise seed hade significantly heavier BW, higher average BWG, better FCR and higher production index compared control birds. Mahmood et al. (2014) found significant improvement of weight gain of broilers fed 0.5 and 1 gm anise seed and improved FCR for birds fed 0.5, 1 and 1.5 anise seed compared to control birds. Al-Kassie et al. (2011) reported that mixture of anise seed and rosemary 0.5%, 0.75% or 1% significantly improved BWG and FCR. Simsek et al. (2007) revealed that anise oil 400 ppm or antibiotics significantly improved body weight of broiler chicks and daily BWG and FCR (Ciftci et al., 2005) compared to control birds. El Deek et al. (2002) concluded that anise at 0.05% improved BWG and FCR significantly however, at 0.1% FCR only was improved. Al-Beitawi et al. (2010) found that, mixture of crushed anise seed, Nigella sativa seeds and Thymus vulgaris as feed additives had significantly improved BW, BWG and FCR of Lohman male broiler chicks compared to control chicks. Alhajj et al. (2015) reported that, 1 and 2 gm/kg Chinese Star Anise significantly improved BW, BWG and feed conversion ratio of Ross 308 broilers. In contrast, Yazdi et al. (2014) found no effect of 1.0 gm anise seed or antibiotics supplementation on BW and FCR of Ross broilers from (0-42 days) compared to control birds. Soltan et al. (2008) concluded that, anise seed supplementation had no effect on FCR. In the same trend, plant extracts or essential oils supplementation had no effect on FCR (Lee et al., 2003; Botsoglou et al., 2004; Hernandez et al., 2004).

The improvement of productive performance could be attributed to anethole and eugenol active ingredient in anise seed which have digestive stimulating factors and destroying microbial pathogens in the alimentary tract (Cabuk et al., 2003). Stimulate digestion of protein, fat and cellulose (Jamroz and Kamel, 2002). Enhance the effects of pancreatic enzymes (Ramakrishna et al., 2003). Enhance liver function, whole tract and ileal digestibility of the nutrients (Hemandez et al., 2004).

3.3 Carcass traits

Effect of dietary supplementation with anise seed and antibiotic on carcass traits of broiler chicks at 43 days of age (Table, 4) revealed that, percentage of carcass weight was significantly higher ($p<0.05$) in anise seed supplemented chicks compared to control birds, with anti-biotic group intermediary but not differed from them ($p>0.05$). Percent of live weight of bursa was significantly higher ($p<0.05$) in anise seed supplemented group compared to control and anti-biotic groups. Percent of live weight of gizzard, heart, and spleen was not significantly differed by dietary treatment. Similar results were reported by Hamodi and Al-Khilani (2012) who reported increased of dressing % of broilers supplemented with anise seed however, the parentage of liver, heart and gizzard was not affected. Zhang et al. (2005) reported improved dressing percent of broilers supplement with essential oil (150 g/ton) compared to control group. Yazdi et al. (2014) found increased bursa % in broilers fed 1gm anise seed compared to control birds however, percentage of dressing, liver, spleen, gizzared and heart was not affected at 1gm anise seed and antibiotic supplementation. Alhajj et al. (2015) found no effect of 1 and 2 gm Chinese Star Anise on the % of lymphoid organs compared to positive (vaccinated) control birds. Soltan et al. (2008) found no effect of anise seed on all carcasses traits however high dose 1.5 gm/kg resulted in significantly reduced % of bursa, spleen and thymus. El-Deek et al. (2002) found no effect of anise, ginger and fennel singly or as a mixture in broiler diets, on dressing percentage and percentage of heart, liver, spleen and pancreas. Simsek et al. (2007) reported significantly increased % of gizzard of broilers fed 400 ppm anise oil compared to control birds however, hot dressing percentage, liver, heart and spleen were not significantly affected. Mahmood et al. (2014) found positive effect of anise seed supplementation on the percentage of spleen weight.

Dietary supplementation had no effect on percentage of bird's mortality (table 4).

3.4 Effect of dietary treatments on differential leukocyte count, Hb content and PCV%.

Table 5. Shows the effects of dietary anise seed and antibiotic supplementation on differential leukocyte count, Hb concentration and PCV%. The current study showed that birds supplemented with 0.5, 0.75, 1.0 gm/kg anise seed or antibiotic had significantly lower heterophil ($p<0.05$) compared to control birds. Similarly, Al-Beitawi et al. (2010) found that, vaccinated broiler chicks fed mixture of crushed Pimpinella anisum, Nigella sativa seeds and Thymus vulgaris leaves supplemented diet had significantly lowered heterophil at 42^{th} day compared to vaccinated unsupplemented birds. Contrarly, Soltan et al. (2008) found that only birds fed 0.5 gm anise seed had significantly lowered neutrophil compared to control birds while other levels had no effect. Lymphocyte was significantly higher in all anise seed and antibiotic supplemented groups compared to control birds. Birds fed .75gm anise had higher lymphocyte compared to antibiotic group. Similarly, Soltan et al. (2008) reported that, anise .25, .5, .75 and 1 gm/kg diet significantly improved lymphocyte compared to control birds. Contrarily, Al-Beitawi et al. (2010) found that, vaccinated male broiler chicks fed herbal mixture including anise had significantly lowered lymphocyte at 42^{th} day compared to vaccinated unsupplemented birds.

H/L ratio was significantly higher in control birds compared to anise fed and antibiotic groups. This result revealed that control group was more stressed. Addition of anise seed or antibiotic to the diet resulted in reduced stress in broiler chicks. This result was in accordance with Maxwell (1993) who reported an increased H/ L ratios in stressed bird that resulted in reduced productivity and immunresponses (Saxena and Madan, 1997).

Dietary supplementation with anise seed had significant effect on Hb concentration. Birds fed 0.5, 0.75 and 1gm/kg anise seed had significantly higher Hb compared to control and antibiotic group. PCV was not significantly affected by dietary treatments. Al-Beitawi et al. (2010) found that, vaccinated broiler chicks supplemented with mixture of anise, Nigella sativa seeds and Thyme leaves had significantly improved Hb at 42^{th} day compared to vaccinate control birds, however HCT was not significantly affected. Contrary to our results, Soltan et al. (2008) revealed that .5 % anise seed significantly improved

Hb concentration and PCV compared to control groups while other levels of anise supplementation had no effect.

3.5. Effect of dietary treatments on fear responses (tonic immobility test- TI).

Dietary supplementation with anise seed and antibiotic had significant effect on the duration of tonic immobility (Table 6). The unsupplemented birds showed significantly longer duration in TI test which indicated that control birds were more fearful compared to anise supplemented and antibiotic groups. Jones (1986) indicated that a long duration of TI is thought to be indicative of high levels of fearfulness. Poor productive performance of control birds may be a consequence of fearfulness. Jones (1996) and Faure et al. (2003) indicated a negative correlation between the welfare and performance of birds and fear responses.

3.6. Immune response.

Effect of anise seed and antibiotic supplementation on antibody titer against NDV (table 7) revealed that antibody titer at 33 days of age was not affected by dietary treatment. This finding was agreed with Soltan et al. (2008) who found that antibody titer against NDV at 34 days of age were not affected by anise seed supplementation. Al-Beitawi et al. (2010) reported that, antibody titer against NDV at 21^{th} and 42^{th} day of age were not significantly differed between vaccinated broiler chicks supplemented with Pimpinella anisum, Nigella sativa seeds and Thymus vulgaris leaves compared to vaccinated unsupplemented birds. Alhajj et al. (2015) indicated that 1 and 2 gm Chinese Anise Star had no effect on antibody titer against NDV compared to vaccinated unsupplemented birds. Contrary, Al-Beitawi et al. (2009) mentioned that, replacement of anise seed to antibiotics resulted in increased the NDV antibody titers compared to control birds at 42 days of age. Also, Bayram et al. (2007) found significantly ($P<0.05$) increased antibody levels against NDV by anise seed supplementation to laying quail diets 5gm per kg. In the same trend, Sirvydis et al. (2003) revealed that the phytobiotics strengthen the immune system and or have antimicrobial properties.

It conclusion anise seed supplementation 0.5, 0.75 and 1gm per kg diet improved feeding and comfort behaviors, productive performance and welfare (reduced fear and stress) of broiler chicks however, immune response was not affected. Anise seed could be used as alternative to antibiotics.

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