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Effects of Ammonia and Nitrite-Nitrate Concentrations on Thyroid Hormones and Variables Parameters of Broilers in Poorly Ventilated Poultry Houses

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Abstract: It was aimed to evaluate the effects of accumulated ammonia (NH_3) concentration in poultry housing and Nitrite (NO_2^-) Nitrate (NO_3^-) concentrations in poultry litter on thyroid hormone levels (Triiodothyronine (T_3) and Thyroxine (T_4)), Body Weight (BW) and variables parameters such as blood methemoglobin, serum retinol, β-carotene and total cholesterol levels. Weighing 58.0±3.2 g (control group) and 60.0±4.3 g (experiment group), 1 day old, 180 male broiler chickens were used. Chicks were allowed *ad libitum* access to feed and water throughout the 45 days trials. In the experiment group, the ventilation was restricted without changing other conditions. NH_3 concentration in poultry housing and moisture ratio, as well as NO_2^--NO_3^- concentrations in litter were measured with 5 days intervals throughout the 45 days trials. Plasma total T_3, T_4, blood methemoglobin and serum retinol, β-carotene and total cholesterol levels were evaluated at the 45 days. NH_3 concentration in poultry housing was increased after 21 days ($p<0.05$) at 0.222 g bird^{-1} day and 26 days ($p<0.01$) at range 0.377-0.400 g bird^{-1} day in the experiment group as compared with the control group throughout the 45 days trials. In addition, moisture ratio in litter were increased after 26 days ($p<0.05$) at 0.444% bird^{-1} day and 36 days ($p<0.01$) at range 0.461-0.472% bird^{-1} day. Also, NO_2^- concentrations in litter were increased after 26 days ($p<0.05$) at range 7.50-8-40 ppm bird^{-1} day. As to NO_3^- concentrations in litter in the experiment group, no statistically significant difference was observed. Compared to control group, at 45 days, BW, plasma total T_3, as well as serum retinol and β-carotene levels decreased significantly in experiment group ($p<0.01$). Total cholesterol level was increased ($p<0.05$). No statistically considerable differences were found in plasma total T_4 and blood methemoglobin levels.

Key words: Ammonia, biochemical parameters, broiler, nitrate, nitrite, thyroid hormone

INTRODUCTION

Ammonia (NH_3) emission is the major concern for poultry operations. Concern about NH_3 in poultry production is not new. Traditionally, the concern has been with the levels of NH_3 inside a poultry house (Pescatore *et al*., 2005; Kim and Choi, 2009). In practice, poultry are often exposed to 50 ppm NH_3. This concentration may rise markedly in poorly ventilated houses, where NH_3 may exceed 200 ppm (Carlile, 1984; Beker *et al*., 2004). It has been suggested that NH_3 should not exceed 25 ppm in poultry houses (Carlile, 1984; Ritz *et al*., 2004). Exposure to 20 ppm for long periods of time has resulted in a variety of disorders, including increased respiratory tract damage and secondary infections such as Newcastle disease, airsacculitis, coccidiosis (Beker *et al*., 2004; Pescatore *et al*., 2005) and *Escherichia coli* infections (Nagaraja *et al*., 1984). Decreased vaccination efficacy has also been related to NH_3 (Caveny *et al*., 1981a). Data further suggest that lung disease as well as inhalation of airborne irritants such as NH_3 result in reduce pulmonary gas exchange and as a result could exacerbate ascites (Beker *et al*., 2004). Results of other studies suggest a relationship between ascites and hypothyroidism, a low heat production per metabolic weight (a low metabolic rate) (Scheele *et al*.,

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1992, 2003; Buys *et al*., 1999; Detailed research Gonzales *et al*. (1999) with 7 male broiler strains also indicated that an altered thyroid hormones Triiodothyronine (T_3) and Thyroxine (T_4) metabolism might increase the bird's susceptibility to ascites. As well as the highest mortality rate due to ascites showed also decreased concentrations of thyroid hormones in plasma (Scheele *et al*., 2003).

In addition, fasted or restricted birds and poorly ventilated represents a permanent stress for any organism. This is of particular concern for young chicks in a phase of rapid growth with relatively high metabolic requirements (Beker *et al*., 2004; Rajman *et al*., 2006). As a result, the entire spectrum of metabolic processes occurs. Many metabolic hormones mediate adaptive changes to physiological stress. Previous research in poultry showed that feed diet deficient or excesses and fasted or restricted modified the plasma levels of hormones that modulate energy metabolism and growth, such as T_3, T_4, growth hormone, insulin-like growth factor-I (Carew *et al*., 1997, 1998; Decuypere *et al*., 2005; Moravej *et al*., 2006; Rajman *et al*., 2006), but this modified may not be in long time continue chronic effects (e.g., stress, poorly ventilation, metabolic disorders).

Thus, NH_3 and Nitrite (NO_2^-) Nitrate (NO_3^-) concentrations in poorly ventilated poultry houses are very important in terms of economic concerns and health problems of workers and birds on the farm. Although, the amount of NH_3 in poultry houses is still of concern, NH_3 emissions are increasing in importance. Information is needed to ascertain consequences under practical low-exposure NH_3 concentrations and its effects in poultry. Therefore, the objective of this study was to evaluate the determine the effects of accumulated NH_3 concentration in poultry housing and NO_2^--NO_3^- concentrations in poultry litter on thyroid hormone (T_3 and T_4) levels, body weight and variables parameters, such as blood methemoglobin, serum retinol, β-carotene and total cholesterol levels.

MATERIALS AND METHODS

Birds, housing and diets: These studies included 90 control group, weighing 58.0±3.2 g and 90 experiment group, weighing 60.0±4.3 g, on average 1 day old male broiler chickens (*Gallus gallus var. domesticus*) with similar experimental designs. In each group, broiler chickens were housed in electrically heated, battery brooders with raised wire floors. They were exposed to a light: dark cycle of 16 h light: 8 h dark and had free access to feed and water. During the experiment, all chicks diets were fed from 1-9 days of age chicks a broiler chick starter diet, from 10-25 of age chicks a broiler grower diet-I and from 26-45 of age chicks a broiler grower diet-II (Table 1). In the control group, brooding temperature in the batteries was set at 35°C for the first week and this temperature was decreased incrementally to 22°C±1.0 by the time the birds were 21 days old and humidity in the room was allowed to fluctuate with changes in ambient temperature. In the experiment group, the ventilation was restricted without changing other conditions. All studies with animals described herein were reviewed and approved by the University of Ankara Institutional Animal Ethics Committee and national regulations.

Table 1: Ingredients and chemical analyses of the starter, grower-I and grower-II diets fed to broilers

Ingredients	Starter diet (%)	Grower diet-I (%)	Grower diet-II (%)
Corn	52.77	53.96	58.62
Soybean meal	41.21	38.06	33.71
Vegetable oil	2.30	4.60	4.59
Limestone	1.18	1.10	1.10
Dicalcium phosphate	1.95	1.70	1.60
DL-methionine	0.14	0.10	0.04
Lysine	0.10	-	-
Sodium chloride	0.25	0.25	0.25
Vitamin-Mineral mix[1, 2]	0.30	0.30	0.30
Calculated analysis, unit			
ME [3] (kcal kg^{-1})	3050	3200	3200
Crude protein (%)	23.00	20.00	20.00
Calcium (%)	1.00	0.90	0.90
Available phosphorus (%)	0.50	0.45	0.45

[1]The vitamin mix provides the following (per kg of diet): 15.000 IU trans-retinyl acetate, 5.000 IU cholecalciferol, 100 mg DL-α-tocopheryl acetate, 100 mg ascorbic acid, 25 mg niacin, 5 mg menadione Na-bisulfite, 3 mg thiamine mononitrate, 6 mg riboflavin, 5 mg pyridoxine HCl, 0.03 mg cobalamin, 1 mg folic acid, 0.2 mg day-biotin, 12 mg Ca-d-pantothenate. [2]The mineral mix provides the following (per kg of diet): 105 mg manganese, 84 mg iron, 84 mg zinc, 9 mg copper, 1 mg iodine, 0.2 mg cobalt, 0.18 mg selenium, 1.04 mg molybdenum. [3]ME: Metabolizable Energy

Sample collection and biochemical analysis: All the poultry litter and feed samples were taken with 5 days intervals during the 45 day experimental period. The samples were taken from 5 different places. Ammonia concentrations in poultry housing were measured using AOAC (1997) procedures. Moisture ratio in poultry litter was performed by drying 2 g of litter and feed at 135°C for 2 h and then weighing it AOAC (1997). In addition, poultry litter samples were prepared for Nitrite (NO_2^-) and Nitrate (NO_3^-) analysis. NO_2^- and NO_3^- concentrations in these samples were determined by the colorimetric method of Sen and Danoldson (1978).

At the end of the study, blood samples were collected into test tubes containing heparin as anticoagulant by from fifteen birds randomly chosen from each groups for plasma total T_3, T_4 and blood methemoglobin levels and centrifuged at 1800 g. In addition, blood samples were collected into nonheparinized-tubes immediately and serum was

separated from blood by centrifugation at 1800 g in order to measure total cholesterol, retinol and β-carotene levels. The plasma and serum samples were frozen at -20°C in aliquots until used.

Plasma total T_3 and T_4 levels were analysed by Radioimmunoassay (RIA) method using RIA kits (Amersham International Ltd., Amersham, United Kingdom). Blood methemoglobin levels were determined by a spectrophotometer (Microlab 200, Merck, Holland) at 630 nm (Fairbanks and Klee, 1987). Serum total cholesterol levels done according to Aras and Ersen (1975). Serum retinol and β-carotene levels were performed by a spectrophotometer (Shimadzu UV-1600, Japan) the method of Suzuki and Katoh (1990).

Statistical analysis: All data were expressed as mean±SE. The statistically significance of differences between the two study groups were determined by means of Student's t-test. $p<0.05$ was set as the limit of significance.

RESULTS AND DISCUSSION

The measured concentrations of NH_3 in poultry housing during the experimental period are given in Table 2. Compared to control group, NH_3 concentration in experiment group showed an increase in NH_3 emissions with broiler age ranging from 0.222 g bird^{-1} day on day 21 ($p<0.05$) to 0.400 g bird^{-1} day on day 45 ($p<0.01$). This is in a reasonably consistent but somewhat higher or lower than the range reported in other recent studies that the NH_3 emissions ranged from 0.027-2.17 g bird^{-1} day with an average of 1.18 g bird^{-1} day (Siefert *et al*., 2004), from 0.213-0.444 g bird^{-1} day (Koerkamp *et al*., 1998), from 0.71-2.34 g bird^{-1} day (Pescatore *et al*., 2005), from 0.024-0.039 g bird^{-1} day (Casey *et al*., 2005) on broilers. Hayes *et al*. (2006) stated that NH_3 emission rates of 0.16, 0.30 and 0.50 g bird^{-1} day were measured for three different broiler units. Lacey *et al*. (2003) reported NH_3 emissions ranging from 0.05-1.90 g bird^{-1} day with an average of 0.63 g bird^{-1} day for broilers over a 49 days growth cycle. Differences in the emission rates from poultry houses may be attributable to seasonal effects, bird ages, litter management, building ventilation rates and the crop day period when the monitoring took place, feed and other process factor along with differences in methodology. As noted by the National Research Council (NRC, 2003), further research is needed to determine how these process factors affect emissions.

In addition to NH_3, although generally there was no difference in ammonia emissions related to litter type or amount used (Elwinger and Svensson, 1996), it has been reported qualitatively that wet litter can lead to high ammonia concentrations in broiler housing (Elliott and Collins, 1982; Kim and Choi, 2009) and may cause bird health problems such as hock burn (Tucker and Walker, 1992). Carr *et al*. (1990) commended that it is desirable to maintain litter moisture below 30% for ammonia control. Elwinger and Svensson (1996) determined that the dry matter content is 91.6-92.2% for fresh litter materials and is about 64% at 35 days of age. In this study, although moisture ratio (%) in the litter calculated during the experiment varied between 29 and 37.5% for initial to 25 days of age ($p>0.05$) and from 33-42.5% for 26-45 days of age ($p<0.05$, $p<0.01$, respectively) (Table 2). Although, these moisture ratios in litter are not high, moisture, in conjunction with high temperature, promotes bacterial growth, which will decompose organic material producing NH_3 in the process. Because NH_3 production is so intimately linked to litter moisture, it is quite difficult to separate the effects of each of these two factors. The combination of NH_3 and wet litter is responsible for a large number of health and density-related welfare problems in poultry. For example, the occurrence of asides, gastrointestinal irritation and respiratory diseases has been correlated with high levels of NH_3. As NH_3 is generated by microbial activity

Table 2: Ammonia concentrations in poultry housing and moisture ratio in litter samples which taken with 5 days intervals during the experimental period of broilers

	Ammonia concentrations					Moisture ratio				
	Control		Experiment			Control		Experiment		
Age (day)	Min.-Max. (g m^{-3})	Mean (days g bird^{-1})	Min.-Max. (g m^{-3})	Mean (days g bird^{-1})	p-value	Min.-Max. (%)	Mean (days % bird^{-1})	Min.-Max. (%)	Mean (days % bird^{-1})	p-value
Air						**Litter**				
Initial	0.0-0.6	0.006	0.0-6.0	0.066	NS	32.5-36.0	0.400	29.0-37.0	0.411	NS
5-10	0.6-12	0.133	6.0-11	0.122	NS	30.0-33.0	0.370	29.0-38.0	0.422	NS
11-15	10-12	0.133	11-12	0.133	NS	31.5-34.0	0.380	30.0-36.5	0.405	NS
16-20	11-12	0.133	12-14	0.155	NS	31.5-33.0	0.370	31.0-37.0	0.411	NS
21-25	10-11	0.122	14-20	0.222	*	32.0-33.0	0.370	31.5-37.5	0.419	NS
26-30	6.0-11	0.122	20-34	0.377	**	31.0-33.0	0.370	33.0-40.0	0.444	*
31-35	6.0-13	0.144	34-38	0.422	**	30.0-32.5	0.360	32.5-40.0	0.444	*
36-40	7.0-13	0.144	34-35	0.388	**	31.0-33.5	0.370	32.5-41.5	0.461	**
41-45	7.0-13	0.144	35-36	0.400	**	31.5-34.5	0.380	33.0-42.5	0.472	**

Min.-Max.: Minimum-Maximum, *: $p<0.05$, **: $p<0.01$, NS: Not Significant

Table 3: Nitrite and nitrate concentrations in litter samples which taken with 5 days intervals during the experimental period of broilers

	Litter									
	Control (Nitrite)				Experiment (Nitrite)				p-value	
Age (day)	Min.-Max. (ppm)	Mean (day ppm bird^{-1})	Min.-Max. (ppm)	Mean (day ppm bird^{-1})	Min.-Max. (ppm)	Mean (day ppm bird^{-1})	Min.-Max. (ppm)	Mean (day ppm bird^{-1})	Nitrite	Nitrate
Initial	2.0-2.1	0.023	499.3-522.3	5.80	2.0-2.1	0.023	502.3-524.4	5.83	NS	NS
5-10	2.1-2.4	0.027	514.5-534.4	5.94	2.3-3.0	0.033	526.7-556.4	6.18	NS	NS
11-15	2.1-2.9	0.032	533.4-536.3	5.96	2.5-3.3	0.037	589.6-595.5	6.62	NS	NS
16-20	2.2-3.1	0.034	532.4-542.5	6.03	2.9-3.6	0.040	602.5-603.6	6.71	NS	NS
21-25	2.4-2.5	0.028	541.4-589.3	6.55	3.0-3.8	0.042	623.4-634.6	7.05	NS	NS
26-30	2.7-2.9	0.032	589.2-594.4	6.60	3.2-4.0	0.044	656.9-675.4	7.50	*	NS
31-35	2.7-2.8	0.031	592.3-602.7	6.70	3.7-4.4	0.049	696.8-699.2	7.77	*	NS
36-40	2.8-3.2	0.035	602.7-609.3	6.77	4.1-4.6	0.051	713.4-715.2	7.95	*	NS
41-45	3.2-3.9	0.043	609.3-622.5	6.92	4.5-5.0	0.055	745.7-756.5	8.40	*	NS

Min.-Max.: Minimum-Maximum, *: $p<0.05$, NS: Not Significant

Table 4: Comparison of plasma, blood and serum parameters including body weight of control and experiment groups in broilers

	Groups		
Parameters (unit)	Control	Experiment	p-value
Body weight (g)			
Initial	58.0±3.2	60.0±4.3	NS
45 days	2068±53.7	1679.3±48.8	**
T_3 (ng dL^{-1})	238±21.6	105.9±6.0	**
T_4 (ng dL^{-1})	0.6±0.1	0.6±0.2	NS
Methemoglobin (Thb[2] %)	0.5±0.2	0.4±0.1	NS
Retinol (μg dL^{-3})	40.6±2.9	25.5±2.3	**
β-carotene (μg dL^{-3})	134±7.9	81.4±6.2	**
Total cholesterol (mg dL^{-3})	158.8±5.8	177.5±2.3	*

Values indicate [1]plasma, [2]blood and [3]serum of the poultry at the end of the 45 days experimental period. *: $p<0.05$, **: $p<0.01$, NS: Not Significant

on faecal uric acid when the litter is moist; therefore any factor affecting litter moisture and manure production will also affect the rate of NH_3 within the house (Liu *et al*., 2006, 2007; Kim and Choi, 2009). In this study, after 20 days, NH_3 concentrations began to increase as moisture contents increased. At day 20, the NH_3 concentration was 14 ppm with litter moisture content of 37%.

At day 35, the NH_3 concentration reached the highest value, 38.0 ppm, with a litter moisture content of 40%. It suggested that moisture increased to litter had an effect of suppressing NH_3 emissions in the short term; however, after a longer time, higher moisture contents in litter eventually resulted in higher NH_3 emissions. It was also noticed that when litter moisture contents were 35% or higher, even after a long time, NH_3 concentrations began to decrease as moisture contents further increased. Elliott and Collins (1982), Carr *et al.* (1990), Groot Koerkamp *et al.* (1995), Kim and Patterson (2005) and Kim and Choi (2009) have reported that wet litter can lead to high NH_3 levels in broiler houses as well as the decrease in NH_3 concentrations at high moisture levels.

This study showed that samples taken in first 3 weeks periods, although NO_2^- and NO_3^- concentrations in both group were not significantly difference in litter ($p>0.05$), but NO_2^- concentrations (from 3.2-5.0 ppm) in litter increased from 4-6 week in experimental group and significant ($p<0.05$) (Table 3). After 4 weeks NO_2^- and NO_3^- concentrations increased and this increase may be related to oxidize NH_3 and poultry manure increases. Nodar *et al.* (1990) and Lehninger *et al.* (1993) also reported that a large portion of NH_3 is oxidized to NO_2^- and eventually to NO_3^- by soil-nitrifying bacteria which there are some nitrifying bacteria in poultry manure. Similarly, Kim and Patterson (2006) reported that if soil-nitrifying bacteria were increased in poultry manure, NH_3 volatilization could be reduced by converting NH_3 to NO_2^- or NO_3^- and fortification would accelerate transformation of NH_3 to NO_2^- or NO_3^- in manure, reducing NH_3 volatilization. In the present study there were significant correlations between NH_3 levels and NO_2^--NO_3^- concentrations after 3 and 4 weeks in experimental group ($p<0.05$).

Although, the effects of dietary on thyroid function have been studied in chickens, nothing is known about the effects of NH_3 level and NO_2^- or NO_3^- concentrations. It was reported previously that the mechanism of the growth depression is not a single mechanism related to decrease in feed intake but is also due to other causes that vary with environmental problems (such as building ventilation, temperature, stress), metabolic and haematological effects (Carew *et al*., 1998; Whyte, 2002; Scheele *et al*., 2003; Beker *et al*., 2004). The results of the present study indicate that plasma T_3 level was found to be 238.0 and 105.9 ng dL^{-1} at 45 days in control and experiment groups, respectively. Also, body weight was 1679.3 g and plasma T_3 level and body weight were decreased significantly by 55.5 and 18.8% ($p<0.01$) for the 36 ppm NH_3 in experiment group at 45 day, respectively (Table 3). It is well known that thyroid activity is important in controlling metabolic rate (Moravej *et al*., 2006). Similarly, there are some reports regarding the relationship between dietary energy and protein and their subsequent effects on performance and intermediary

metabolism (Carew *et al*., 1997, 1998; Gonzales *et al*., 1999; Darras *et al*., 2000; Khazali and Moravej, 2003; Moravej *et al*., 2006). Several factors could lead to low plasma T_3 levels in experiment group. First, secretion rate and activity of the thyroid gland may be decreased as NH_3 concentration increased in houses. Second, there could be higher clearance of T_3 from the blood. Third, the low retinol level in these broilers may be linked to consumption of other antioxidant vitamins (vitamin C and E) as a result of increased oxidative stress related to NH_3 concentration increased in poultry housing. Also, retinol deficiency in chicks may be occur hypothyroidism.

In addition, some studies suggested a change in the rate of conversation of T_3 to T_4 between starting, growing and finishing chickens (Williams and Njoya, 1998; Decuypere and Buyse, 2005). Generally, it has been reported that a reduction in plasma T_3 is accompanied by an increase in T_4 as a result of a reduction in peripheral monoiodination of T_4 (Moravej *et al*., 2006). However, in the current study the results of plasma T_4 levels was determined be 0.6 ng dL^{-1} in both group at 45 days and plasma T_4 levels was not significantly different between control group ($p>0.05$) (Table 4). Similarly Carew *et al*. (1997, 1998, 2005) reported that plasma T_4 levels were generally resistant to change and may be change in plasma T_3 levels without accompanying changes in plasma T_4 level. In addition, usually, reductions in growth and weight gain by depress plasma levels of T_3 or poorly ventilated houses (Caveny *et al*., 1981b; Carlile, 1984; Carew *et al*. 1998, 2003; Beker *et al*. 2004; Decuypere and Buyse, 2005; Pescatore *et al*., 2005; Moravej *et al*., 2006; Rajman *et al*., 2006). This effect was also observed in the study, compared to control, the body weight decreased (from 2068-1679 g) at 45 days in the experiment group as related to NH_3 increases and hypothyroidism occurred ($p<0.01$) (Table 3) and may explain the depressive effects of NH_3 concentration growth rate and metabolism. Beker *et al*. (2004) found similar body weight and weight gain-related variation by NH_3 increased in broiler chicks and reported that body weight and weight gain were decreased by 3.5 and 2.5% for the 30 ppm NH_3 at 21 days, respectively. Although, changes in thyroid hormone metabolism are known to affect growth, Rosebrough *et al*. (1999) and Carew *et al*. (1998) shown that reductions in blood T_3 levels were depressed growth, but not related to changes in feed intake.

In this study, compared to control group, serum retinol and β-carotene levels in the experimental group at 45 days were decreased and found to be 25.5 and 81.4 μg dL^{-1}, respectively ($p<0.01$), but total cholesterol level was increased and at 177.5 mg dL^{-1} ($p<0.05$) and blood methemoglobin level was unchanged and at 0.4% THb ($p>0.05$) (Table 4). Similarly, Smolle *et al*. (1983) reported that one factor that affects serum retinol is hyperthyroidism and hypothyroidism, the serum levels of carotene in hypothyroidism only. Nockels *et al*. (1984) and Spear and Moon (1986) also showed that hypothyroidism is an early sing or predispose of retinol deficiency in chicks. As well as, total serum cholesterol concentrations have generally been found to be elevated in human patients with hypothyroidism (Becker, 1986). Also, reducing feed intake or feed restriction and/or growth period significantly increased plasma cholesterol (Hollands *et al*., 1980; Carew *et al*., 2003; Rajman *et al*., 2006; Dikmen and Sahan, 2007) and the inverse correlation between serum levels of cholesterol and thyroid hormone has been known from clinical studies (Marino *et al*., 1984; Shin and Osborne, 2003). Peebles *et al*. (1997) found similar age-related variation in cholesterol metabolism in broiler chicks. Also, modeling physiological stress in chickens showed that metabolic changes associated with stress in chicken are increased plasma cholesterol level (Puvadolpirod and Thaxston, 2000a, b) as it was determined in serum in this study. Although, there were limited data on the correlation investigations between retinol and diodinase enzymes in avian species, Darras *et al*. (2006) reported that like mammals and most other vertebrates, birds possess three types of iodothyronine deiodinases (D_1, D_2 and D_3) that closely resemble their mammalian counterparts, as shown by biochemical characterization studies in several avian species. Deiodination in birds is subject to regulation by hormones from several endocrine axes, including thyroid hormones and growth hormone. Also, deiodination is also influenced by external parameters, such as nutrition, temperature, light and also a number of environmental pollutants (Darras *et al*., 2006).

CONCLUSION

The hypothesis of the present study was that NH_3 increased as depending moisture ratio and poorly ventilation in housing in rearing periods in broilers may lead to stress and retinol level in these animals could decreased by linked to consumption of other antioxidant vitamins as a result of increased oxidative stress related to NH_3 concentration increased. Also, antioxidant vitamins deficiency such as retinol may be triggering several events such as body weight or weight gain loss and metabolic disorders (e.g., such as hypothyroidism, dyslipidemi) and hepatic enzyme inhibition. Supplementing a combination of antioxidant vitamins and minerals may offer a potential protective management

practice in preventing stress related poorly ventilation in rearing performance of broiler. Furthermore, any efforts to reduce ammonia levels and litter management will have a large impact on the health, welfare and performance of the chickens.

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