Effect of oxygen supplementation in a hatchery at high altitude and growth performance of broilers reared at low altitude

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Abstract

The objective of this study was to investigate the effect of oxygen supplementation on broiler eggs in a hatchery at high altitude on the growth performance and ascites syndrome of broilers reared at low altitude. The treatment groups were low altitude with no oxygen supplemented in the hatchery (LA-NOX); high altitude with oxygen supplementation in the hatchery (HA-OX); and high altitude with no oxygen supplemented in the hatchery (HA-NOX) group. Growth performance, heart weight, the concentrations of the hormones, T₃, T₄, T₃/T₄, and and plasma concentrations of haematocrit, haemoglobin, glucose and parameters of ascites syndrome during the growing period were investigated. A total of 243 one-day-old broilers were used for this study. During the growing period, excluding days 7, 28 and 35, oxygen supplementation at high altitude did not affect the live weight of broilers compared with the HA-OX and HA-NOX groups. The cumulative feed consumption was determined to be lower in the LA-NOX group and the same in the HA-OX and HA-NOX groups on the 42nd day. Between 21 and 42 days old, the LA-NOX group had a better feed conversion ratio (FCR) than the HA-OX and HA-NOX groups. Chick weight (CW), yolk sac weight (YSW) and chick heart weight (CHW) were higher in the LA-NOX group than in the HA-OX and HA-NOX groups. At 42 days old, there were no differences between the groups in heart weight, right ventricle weight (RV), left ventricle and septum (LV+Sept.), total ventricle (TV) weight and the RV : TV ratio. The plasma T₃ level was lower in the LA-NOX group than in the HA-OX and HA-NOX groups and T₄ levels were higher in the HA-OX than in the others at 42 days old. The hypoxic conditions that occurred during the embryonic stage - which altered endogenous functions of prenatal chicks and affected several blood parameters, and oxygen supplementation at high altitude - improved chick quality. However, it did not improve subsequent FCR and feed consumption performance of chickens when they were reared at low altitude.

Keywords: Breeder eggs, growth, high altitude, oxygen supplementation [#]Corresponding author: bilgehan@uludag.edu.tr

Introduction

The partial pressure of oxygen (O_2) becomes lower with increasing altitude (Visschedijk, 1985) and a decrease in barometric pressure and O_2 partial pressure at high altitude causes a lack of O_2 (hypoxia), carbon dioxide (CO_2) (hypocapnia) and water (dehydration) in chickens (Visschedijk, 1991). Growth increases the need for O_2 consumption (Beker *et al.*, 2003), and rapidly growing broiler chickens need O_2 for their high metabolic requirements (Julian *et al.*, 1989). As the O_2 level decreases with altitude, exposure to chronic hypoxia increases mortality during incubation and decreases growth as a result of adaptation (Julian, 2000; Villamor *et al.*, 2004). The hypoxic condition during incubation decreases chick weight (Dzialowski *et al.*, 2002; Sharma *et al.*, 2006; Zhang *et al.*, 2008). However, Bahadoran *et al.* (2010) found that bodyweight of newly hatched chicks from a high-altitude incubator was significantly higher than that of chicks incubated in a low-altitude one. Additionally, Giussani *et al.* (2007) showed that O_2 supplemention of eggs incubated at sea-level could prevent high-altitude induced growth restriction completely. However, Çelen *et al.* (2009) found no positive impact of additional O_2 supplementation at high altitude during the incubation period on weight of chicks from 31 and 55 weeks of age in broiler breeders. Meshew (1949) found that O_2

supplementation to the hatchery at high altitude resulted in a slightly higher hatching weight of chicks and turkeys than no supplemented incubation.

Previous studies suggested that O_2 and CO_2 exchanges are important for embryonic development during incubation and especially for chick embryo survival (Tona *et al.*, 2005; Altan *et al.*, 2006; Şahan *et al.*, 2011). At high altitude, chronic hypoxia is a more serious problem during incubation, because it affects chick embryo survival and hatchability, and has detrimental consequences for bodyweight and post-hatch bird performance (Decuypere *et al.*, 2001; Chan & Burggren, 2005).

This could be because of decreased efficiency in foetal resource uptake from the yolk sac, which is converted to embryonic tissues, thus making it unusable (Esmail, 2012). The importance of the yolk sac is not restricted to the life of the embryo in avian species, but extends to post-hatch life (Romanoff, 1960). Inefficient foetal resource uptake from the yolk sac increases the incidence of ascites, which has become of increasing concern (Silva *et al.*, 1988), because of its association with increased mortality and decreased weight gain (Julian, 1993). Oxygen concentration plays a major role in the onset of ascites; reduction in atmospheric O_2 concentration effectively induces ascites. It is therefore important to keep the O_2 concentration above 19.6% to minimize ascites-related anomalies and maximize performance (Beker *et al.*, 2003). Fast-growing chicken breeds are more likely to suffer from ascites because of their rapid growth and high metabolic rate, both of which require more O_2 (Acar *et al.*, 1995). The peak of ascites incidence occurs during weeks 5 to 6 of the growing period (Coleman & Coleman, 1991).

A higher metabolic rate is associated with increased secretion of the hormones, thyroxine (T_4) and triiodothyronine (T_3), which are important growth promoters in chickens (Gonzales *et al.*, 1999; Yahav, 2000; Luger *et al.*, 2001). Thyroid hormones regulate the metabolic rate during the post-hatch period (Gabarrou *et al.*, 1997; Decuypere *et al.*, 2000) and are linked with ascites susceptibility (Hassanzadeh *et al.*, 2004; De Smit *et al.*, 2005). This becomes even more apparent under adverse environmental conditions, such as high altitude (Hassanzadeh *et al.*, 2004).

Before a bird exhibits gross ascites syndrome lesions, commonly the right ventricle to total ventricle (RV : TV) ratio, changes in the concentrations of haemoglobin (Hb), haematocrit (PCV) (Yersin *et al.*, 1992; Yahav *et al.*, 1997; Wideman *et al.*, 1998), glucose in the liver (Diaz-Cruz *et al.*, 1996), blood gases and other parameter changes can be detected (Huchzermeyer & DeRuyck, 1986; Maxwell *et al.*, 1986; 1987). It is generally accepted that the greater right ventricle to total ventricle ratio (RV : TV) (0.29 vs. 0.20) is an indication of ascites (Julian, 1993; Owen *et al.*, 1995; Wideman, 2001).

In previous research, Şahan *et al.* (2011) demonstrated that O_2 supplementation at high altitude during the late incubation period of broiler eggs increased hatchability and embryonic survival rates because of decreased hypoxic stress. In this study, the aim was therefore to investigate the effect of high-altitude O_2 supplementation on growth performance and ascites susceptibility of broiler chicks reared at low altitude.

Material and Methods

The housing and experimental procedures reported in this experiment were carried out according to the Institutional Animal Care and Use Committee of Uludag University of Bursa, Turkey. During the experiment, all researchers had valid animal care and use certificates. The fertile eggs were obtained from a commercial broiler breeder parent stock (Ross 308) at 50 weeks old, and reared at low altitude (100 m). The experimental details of incubation period in this study followed the method described by Şahan *et al.* (2011).

At hatch, 243 chicks from each treatment group were randomly selected, and weighed for the second part of the trial. Chicks from each treatment group were randomly divided over three floor pens (27 chicks per pen, each with three replicates) and reared on a low-altitude farm (100 m above sea level). The same trial groups – LA-NOX (low altitude with non-oxygen supplemented in the hatchery), HA-NOX (high altitude with non- O_2 supplemented in the hatchery) and HA-OX (high altitude with O_2 supplemented in the hatchery) – were used. Wood shavings were used as litter material, and spread to the depth of 7 - 8 cm on the floor. At placement, ten 1-day-old chicks were randomly selected from each treatment group (a total of 30), weighed and killed by decapitation. The heart, liver and yolk sac were removed and weighed with a digital scale with \pm 0.1 g precision for chick weight (CW), heart weight (CHW), yolk sac weight (YSW) and liver weight (LW).

The chicks were reared under a continuous lighting programme, 24 h light from day 1 to day 5, followed by 23 h light and 1 h darkness for the remainder of the trial period. The feeding programme consisted of a commercial standard broiler starter diet (220 g CP/kg and 12.8 MJ ME/kg) fed from day 1 to 14, a grower diet (220 g CP/kg and 13.3 MJ ME/kg) fed from day 15 to 28, and a finisher diet (210 g CP/kg and 13.5 MJ ME/kg) fed from days 29 to 42. Feed and water were provided *ad libitum*. Individual live weight and feed consumption values were recorded and cumulative feed consumption and feed conversion ratio (FCR) were calculated every week for each pen. Mortality was recorded daily. All dead birds were examined for heart failure and ascites lesions, as determined in previous publications (Julian *et al.*, 1989; Julian, 1993).

At the end of the trial, at 42 days of age, 11 broilers were randomly selected from each treatment group and killed by decapitation. The hearts were removed and dissected to obtain heart weights for calculating the right ventricle (RV), total ventricle weights (TV) as well as the RV : TV ratio. The heart weight, right and total ventricles were weighed on a digital scale with \pm 0.1 g precision.

At days 1, 7, 21 and 42, approximately 1 mL blood samples were collected randomly from 15 broilers per treatment group via cardiac puncture into lithium heparinized tubes on ice to separate plasma and determine plasma thyroid hormone (T_3 , T_4 , $T_{3/4}$), PCV, Hb and glucose concentrations. The PCV concentration was determined by centrifuging the blood in heparinized capillary tubes in a micro capillary centrifuge (Nuve Laboratory Equipment, Ankara, Turkey) for 5 min at 13000 × *g* and visualized on a reader (International Equipment Co., Needham, Mass, USA). The Hb concentration was analysed colorimetrically with a Biolabo Reagents diagnostic kit (Biolabo Sa, Maizy, France) according to the manufacturer's instructions. After centrifugation (Hettich EBA 21 Centrifuge, GMI Inc., Minnesota, USA) at 3000 × *g* for 10 min the cellular fraction was separated from the plasma. The plasma was stored at -20 °C until it was analysed by radioimmunoassay (Davis *et al.*, 2000). The T₃ assay was characterized by intra-assay and inter-assay variations (CV) of 2.11% and 3.15%, respectively. The T₄ assay was characterized by intra-assay and inter-assay CV of 1.96% and 4.10%, respectively. The blood glucose was determined by the glucose oxidase method (Sigma Chemical Co.). Chicks were reared under a continuous lighting programme, 24 h light from day 1 to 5, followed by 23 h light and 1 h darkness for the remainder of the trial period.

The data were subjected to one-way analysis of variance (ANOVA) using the general linear models procedure in SAS (2007). The analysis for the percentage data of PCV was conducted after arcsine transformation. Significant differences between the treatment means were determined by Duncan's multiple range test. A probability of P <0.05 was considered significant.

Results

Bird live weight, weekly basis feed consumption, cumulative feed consumption and feed conversion values of the low altitude (LA-NOX), high altitude (HA-NOX) and high altitude with O_2 supplemented (HA-OX) groups are given in Table 1. From the 14th to 28th day of age, live weights were higher in the LA-NOX group than the high altitude groups (P < 0.01) while there was no significant difference at 7 days old (P > 0.05). Also, at days 35 and 42, O_2 supplementation at a high altitude did not affect the live weight of broilers compared with the HA-OX and HA-NOX groups.

In this study, weekly basis feed consumption was found to be significant on the 28th, 35th and 42nd days (P < 0.01). Oxygen supplementation at high altitude affected the weekly basis feed consumption, and the LA-NOX and HA-OX groups were found to be similar at the 28th and 35th days. The lowest weekly basis feed consumption was found in the LA-NOX group on the 42nd day (P < 0.01). The cumulative feed consumption was determined to be lower in the LA-NOX group and the same in the HA-OX and HA-NOX groups on the 42nd day (P < 0.05). The FCR was affected by altitude. Between 21 and 42 days old, the LA-NOX group had a better FCR compared with the HA-OX and HA-NOX groups (P < 0.01). The FCR was the same in both HA-OX and HA-NOX groups at the 21st, 28th, 35th and 42nd days of age.

The chick body and organ weight values and mortality rate of the LA-NOX, HA-NOX and HA-OX groups are given in Table 2. The CW (P < 0.01), YSW (P < 0.05) and CHW (P < 0.01) were higher in the LA-NOX group than the HA-OX and HA-NOX groups. At 42 days old, there were no differences in heart weight, RV, LV+Sept., TV weight and the RV : TV ratio between the groups (P > 0.05). In the present study, total mortality during to growing period did not differ between the groups. A mortality ratio because of ascites was not observed in the groups. However, during the growing period, two mortalities were observed in HA-NOX group (Table 2).

The thyroid hormones and blood parameters of the broilers of the LA-NOX, HA-OX and HA-NOX groups are given in Table 3. The lowest ratio of T_3 : T_4 value was found in the LA-NOX group (P < 0.01). The plasma T_4 level did not differ between groups (P > 0.05). Chick blood PCV value was lower in the LA-NOX group than in the high-altitude groups (P < 0.01). The highest one-day-old chick blood Hb value was found in the HA-NOX group compared with the HA-OX and LA-NOX groups (P < 0.01). The glucose level did not differ between groups (P > 0.05) in one-day-old chicks. However, there were differences in blood glucose level, and the highest blood glucose level was found in the HA-OX group at 7 and 21 days old (P < 0.01). The plasma T_3 level was lower in the LA-NOX group than the HA-OX and HA-NOX groups and T_4 levels were higher in the HA-OX than the others at 42 days old (P < 0.01). The highest PCV value was found in the LA-NOX group at 42 days old (P < 0.05).

Variables	Treatment	Age (day)								
		Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42		
		**	NS	**	**	**	*	*		
Live weight, g	LA-NOX	$42.9^{a}\pm0.55$	145.7 ± 2.57	$340.8^{a} \pm 0.89$	$702.0^{a} \pm 5.45$	1242 ^a ± 5.17	1832 ^a ± 15.50	$2420^{a} \pm 3.32$		
Live weight, g	HA-OX	$40.8^{\text{b}}\pm0.33$	140.3 ± 0.74	325.8 ^b ± 1.58	636.1 ^b ± 12.02	1148 ^b ± 18.86	1657 ^b ± 55.81	2269 ^b ± 45.22		
	HA-NOX	$40.9^{\text{b}}\pm1.18$	140.4 ± 4.54	331.6 ^b ± 2.89	$649.5^{b} \pm 5.10$	$1196^{ab} \pm 0.47$	1720 ^{ab} ± 40.18	2285 ^b ± 29.53		
			NS	NS	NS	** ** **	**			
Weekly basis feed	LA-NOX	-	159.7 ± 0.86	240.4 ± 0.18	398.5 ± 5.33	948.5 ^b ± 19.67	837.4 ^b ± 19.67	1306.3 ^b ± 2.99		
consumption, g	HA-OX	-	152.6 ± 2.28	240.7 ± 1.06	448.5 ± 17.12	949.4 ^b ± 11.01	838.3 ^b ± 11.012	1428.0 ^a ± 3.10		
concernption, g	HA-NOX	-	154.6 ± 1.65	238.9 ± 1.07	413.0 ± 18.38	$1034.0^{a} \pm 4.64$	918.4 ^a ± 2.073	1422.0 ^a ± 13.36		
				NS	NS	*	*	*		
Cumulative feed	LA-NOX	-	-	399.5 ± 0.18	798.0 ± 5.16	$1747^{b} \pm 14.44$	2584 ^b ± 34.19	3890.7 ^b ± 31.20		
consumption, g	HA-OX	-	-	395.1 ± 2.54	843.6 ± 14.58	1793 ^{ab} ± 25.60	2631 ^b ± 36.61	4059.0 ^a ± 33.51		
	HA-NOX	-	-	394.4 ± 0.32	807.4 ± 18.70	1841 ^a ± 14.06	2760 ^a ± 11.99	$4006.0^{a} \pm 20.83$		
			NS	NS	**	**	**	**		
Feed conversion	LA-NOX	-	1.09 ± 0.026	1.18 ± 0.003	$1.14^{b} \pm 0.017$	$1.41^{b} \pm 0.006$	$1.41^{b} \pm 0.029$	$1.61^{b} \pm 0.012$		
ratio, g/g	HA-OX	-	1.08 ± 0.023	1.21 ± 0.011	$1.32^{a} \pm 0.002$	$1.56^{a} \pm 0.003$	$1.60^{a} \pm 0.032$	$1.79^{a} \pm 0.023$		
	HA-NOX	-	1.10 ± 0.049	1.19 ± 0.010	$1.25^{a} \pm 0.020$	1.54 ^a ± 0.011	$1.61^{a} \pm 0.031$	1.76 ^a ± 0.015		

Table 1 Broiler live weight, weekly basis feed consumption, cumulative feed consumption and feed conversion values of groups (mean ± SEM)

^{a,b} Within columns, within variables, means with different superscripts are significantly different at ** *P* <0.01 and **P* <0.05; NS: not significant.

LA-NOX: low altitude, HA-OX: high altitude with oxygen supplemented, HA-NOX: high altitude with non-oxygen supplemented; n: 81 chicks were used for each treatment group (total 243 chicks).

Body part values	n	LA-NOX	HA-OX	HA-NOX	Р
1 d old broiler					
CW, g	10	43.55 ^ª ± 1.47	$40.04^{b} \pm 1.14$	40.37 ^b ± 1.41	**
YSW, g	10	$4.59^{\text{a}} \pm 1.38$	$\textbf{3.40}^{b} \pm \textbf{0.94}$	$\textbf{3.96}^{b} \pm \textbf{0.59}$	*
LW, g	10	$\textbf{1.18} \pm \textbf{0.13}$	1.16 ± 0.07	1.25 ± 0.08	NS
CHW, g	10	$0.39^{a}\pm~0.01$	$0.35^{\text{b}}\pm0.01$	$0.34^{\text{b}}\pm0.01$	**
42 d old broiler					
Heart weight, g	11	$\textbf{8.07} \pm \textbf{0.29}$	$\textbf{7.41} \pm \textbf{0.42}$	$\textbf{7.40} \pm \textbf{0.46}$	NS
Right ventricle (RV), g	11	1.03 ± 0.04	$\textbf{0.92} \pm \textbf{0.05}$	1.02 ± 0.08	NS
Left ventricle(LV) +Sept., g	11	$\textbf{5.87} \pm \textbf{0.19}$	5.01 ± 0.22	5.53 ± 0.38	NS
Total ventricle (TV), g	11	$\textbf{6.90} \pm \textbf{0.21}$	5.92 ± 0.26	$\textbf{6.56} \pm \textbf{0.45}$	NS
RV : TV	11	$\textbf{0.15} \pm \textbf{0.01}$	$\textbf{0.15} \pm \textbf{0.01}$	$\textbf{0.16} \pm \textbf{0.00}$	NS
Mortality rate		-	-	2/81	

Table 2 Chick and organ weight values and mortality rate of groups (mean ± SEM)

^{a,b} Means within rows with no common superscript are significantly differ at $P < 0.01^{**}$ and $P < 0.05^{*}$. NS: not significant.

LA-NOX: Iow altitude; HA-OX: high altitude with oxygen supplemented; HA-NOX: high altitude with non-oxygen supplemented; CW: chick weight; CHW: chick heart weight; YSW: yolk sac weight; LW: liver weight.

Discussion

Depending on the higher incidence of ascites, high-altitude incubated chickens had a different growth pattern during their post-hatch growing period and reached their maximum growth at 6 weeks old (Bahadoran *et al.*, 2010). In the present study, from 14 to 42 days old, live weights were higher in the LA-NOX group than in the high altitude group. The O_2 supplementation at high altitude did not affect the live weight of broilers compared with the HA group except at days 7, 28 and 35. The results of the present study are in contrast with those of Hassanzadeh *et al.* (2004), Bahadoran *et al.* (2010) and Çelen *et al.* (2009), who showed that high-altitude incubated chickens had significantly higher bodyweights than low-altitude incubated chickens at 42 days old. In contrast to our findings, Meshew (1949) and Wilgus & Sadler (1954) report a slightly heavier weight at 2 and 3 weeks old when O_2 was added.

The lipid uptake from the yolk sac between day 22 and hatch was greater in embryos from the faster growing line (Ding *et al.*, 1995). The yolk sac played a critical role in growth of broiler chicks during the first week of age; thereafter the chicks compensated for bodyweight gain (Ali *et al.*, 2007). Similarly, Bhanja *et al.* (2009) found that faster utilization of yolk sac resulted in better weight gain of broilers at 5 weeks old. In the present study, the yolk sac weight was found to be lower in the HA groups than the LA-NOX group, and, in contrast to Bhanja *et al.* (2009), at the end of the growing period, the LA-NOX group had higher live weights than the HA groups.

Some researchers have suggested that extra O_2 supplementation during high-altitude incubation improves body weight, feed consumption and FCR (Beker *et al.*, 2003; Quintana *et al.*, 2006; Çelen *et al.*, 2009) in 42-day-old broilers reared at low altitude. However, in the present study, at the end of the growing period, feed consumption and FCR were lower in the LA-NOX group than the HA ones. However, O_2 supplementation at high altitude did not affect feed consumption and FCR. In contrast to our findings, Hassanzadeh *et al.* (2004) and Bahadoran *et al.* (2010) found that there were no differences between the feed intakes of the high-altitude and low-altitude incubated groups during the growing period, and FCR was found to be lower in the high-altitude incubated group than low-altitude incubated group.

In the present study, altitude affected CHW. However, the O_2 supplementation at high altitude did not affect heart weight. These findings agree with past research (Richards *et al.*, 1991; Christensen *et al.*, 1997). For rapid growth a broiler makes blockades in supply organs such as the liver and the gastrointestinal tract after hatching (Katanbaf *et al.*, 1988). In the present study, altitude affected the chick yolk sac weight; though O_2 supplementation at high altitude did not affect it. Neither altitude nor O_2 supplementation affected chick liver weight.

Broilers		LA-NOX	HA-OX	HA-NOX	Р
1 d old broiler	n				
Plasma T ₃ , ng/mL	15	0.75 ^b ±0.17	$0.85^{ab}\pm0.12$	0.97 ^a ±0.14	**
Plasma T ₄ , ng/mL	15	10.05 ± 0.92	$\textbf{9.82}\pm\textbf{0.89}$	$\textbf{9.15} \pm \textbf{1.34}$	NS
Plasma T ₃ /T ₄	15	$0.07^{\text{c}}\pm0.02$	$\textbf{0.09}^{b} \pm \textbf{0.01}$	$\textbf{0.11}^{a}\pm\textbf{0.01}$	**
PCV (haematocrit), %	15	$27.45^{b} \pm 0.51$	$\textbf{32.50}^{\text{a}} \pm \textbf{1.00}$	$\textbf{30.82}^{\text{a}} \pm \textbf{1.00}$	**
Hb (haemoglobin), g/dL	15	$8.96^{b}\pm0.70$	$\textbf{8.17}^{b} \pm \textbf{0.21}$	$11.63^{a}\pm0.22$	**
Glucose, mg/dL	15	268.30 ± 6.07	256.40 ± 4.77	$\textbf{273.90} \pm \textbf{6.62}$	NS
7 d old broiler					
Plasma T ₃ , ng/mL	15	$\textbf{3.40}\pm\textbf{0.12}$	$\textbf{3.15}\pm\textbf{0.10}$	$\textbf{3.24}\pm\textbf{0.11}$	NS
Plasma T ₄ , ng/mL	15	$\textbf{7.23} \pm \textbf{0.39}$	$\textbf{6.57} \pm \textbf{0.25}$	$\textbf{7.39} \pm \textbf{0.35}$	NS
Plasma T ₃ /T ₄	15	$\textbf{0.50}\pm\textbf{0.04}$	$\textbf{0.49} \pm \textbf{0.03}$	$\textbf{0.45}\pm\textbf{0.03}$	NS
PCV (haematocrit), %	15	$\textbf{25.33} \pm \textbf{0.50}$	25.20 ± 0.40	$\textbf{26.07} \pm \textbf{0.76}$	NS
Hb (haemoglobin), g/dL	15	$\textbf{6.67} \pm \textbf{0.18}$	$\textbf{6.16} \pm \textbf{0.19}$	$\textbf{6.57} \pm \textbf{0.21}$	NS
Glucose, mg/dL	15	$\textbf{250.1}^{b} \pm \textbf{7.13}$	$315.6^{a} \pm 15.64$	$\textbf{254.9}^{b} \pm \textbf{6.70}$	**
21 d old broiler					
Plasma T ₃ , ng/mL	15	2.51 ± 0.08	$\textbf{2.82}\pm\textbf{0.06}$	$\textbf{2.70} \pm \textbf{0.13}$	NS
Plasma T ₄ , ng/mL	15	8.39 ± 0.52	$\textbf{7.93} \pm \textbf{0.50}$	8.57 ± 0.38	NS
Plasma T ₃ /T ₄	15	$\textbf{0.32}\pm\textbf{0.02}$	$\textbf{0.38} \pm \textbf{0.03}$	$\textbf{0.33}\pm\textbf{0.02}$	NS
PCV (haematocrit), %	15	$\textbf{28.20} \pm \textbf{0.60}$	$\textbf{27.53} \pm \textbf{0.43}$	28.40 ± 0.42	NS
Hb (haemoglobin), g/dL	15	$\textbf{7.05}^{b} \pm \textbf{0.26}$	$\textbf{7.87}^{\text{ab}}\pm\textbf{0.13}$	$8.64^{a}\pm0.65$	*
Glucose, mg/dL	15	$\textbf{249.0}^{\text{b}} \pm \textbf{3.40}$	$\textbf{268.9}^{a} \pm \textbf{5.15}$	$\textbf{253.3}^{b} \pm \textbf{3.59}$	**
42 d old broiler					
Plasma T ₃ , ng/mL	15	$\textbf{0.39}\pm\textbf{0.02}$	$0.71^{a}\pm0.03$	$0.65^{a}\pm0.08$	**
Plasma T ₄ , ng/mL	15	$\textbf{12.61}^{b}\pm\textbf{0.63}$	$\textbf{17.05}^{a} \pm \textbf{0.56}$	$13.45^{\text{b}}\pm0.40$	**
Plasma T ₃ /T ₄	15	$\textbf{0.04} \pm \textbf{0.00}$	$\textbf{0.05} \pm \textbf{0.00}$	$\textbf{0.05} \pm \textbf{0.00}$	NS
PCV (haematocrit), %		$\textbf{30.20}^{a} \pm \textbf{0.40}$	$\textbf{29.73}^{ab} \pm \textbf{0.30}$	$28.33^{b}\pm0.59$	*
Hb (haemoglobin), g/dL 15		$\textbf{6.47} \pm \textbf{0.14}$	$\textbf{6.73} \pm \textbf{0.08}$	$\textbf{6.29} \pm \textbf{0.17}$	NS
Glucose, mg/dL 15		194.1 ± 5.52	215.8 ± 5.67	194.8 ± 6.39	NS

 Table 3 Thyroid hormones and blood parameters of broilers at low altitude, high altitude and high altitude in oxygen supplemented groups at different periods (mean ± SEM)

^{a,b,c} Means within the same row with no common superscript are significantly different at $P < 0.01^{**}$ and $P < 0.05^{*}$. NS: not significant.

LA-NOX: Iow altitude; HA-OX: high altitude with oxygen supplemented; HA-NOX: high altitude with non oxygen supplemented;

n: number of broiler.

The RV : TV, haemoglobin, hematocrit, blood gases and specific clinical chemistries can be used to determine the ascites status of a bird before gross lesions are apparent (Huchzermeyer & DeRuyck, 1986). In the present study, neither altitude nor O_2 supplementation affected the heart weight, RV, LV+Sept., TV weight or the RV : TV ratio at 42 days old. The highest RV : TV ratio found was 0.16, which is lower than what is considered an indicator for ascites development (Julian, 1993; Wideman, 2001). The RV : TV ratio had been clarified for broilers previously (Julian *et al.*, 1989; Julian, 1993) and a healthy broiler should have an RV : TV ratio lower than 0.25, but is at risk if this value is greater than 0.29. Mortality because of the ascites syndrome was not observed in our groups. Numerically, two mortalities were observed in the HA-NOX group. In contrast to our findings, Bahadoran *et al.* (2010) found that high-altitude incubated chickens indicated lower RV hypertrophy and greater ascites mortality than low-altitude incubated chickens during the

growing period at high altitude. Similar to our findings, there was no mortality difference in low-altitude reared groups incubated at low or high altitude (Hassanzadeh *et al.*, 2004).

Thyroid hormones are important for regulating the metabolic rate during the post-hatch period (Decuypere et al., 2000; Lin et al., 2008) and are linked with ascites susceptibility in broiler chickens' later life (Hassanzadeh et al., 2004; De Smit et al., 2005). This becomes even more apparent under adverse environmental conditions, such as high altitude (Hassanzadeh et al., 1999; 2004). At high altitude, there is an increase in the blood viscosity accompanied by an increased haematocrit together with pulmonary vasoconstriction (Julian, 1993). In the present study, altitude affected the one-day-old chick plasma T_3 level, T₃:T₄, haematocrit (PCV) and haemoglobin (Hb) values, and O₂ supplementation affected the T₃:T₄ and Hb values. The observed blood plasma Hb in this study is in agreement with findings by Bagley & Christensen (1991), but is in contrast with Hassanzadeh et al. (2004) for plasma T₃, T₄ and T₃: T₄ levels. Similarly, in agreement with Christensen et al. (1997), O2 supplementation during incubation did not affect blood glucose concentration of hatched chicks. The PCV value is a reflection of physiological O₂ transport capacity (lpek & Sahan 2006). However, Luger et al. (2001) suggested that hematocrit and thyroid hormones could provide a good indication of ascites development but only during the last week of life and not in all cases. None of these parameters, however, can predict the development of ascites at an early age. In the present study, during the growing period at low altitude, the highest blood glucose level was found in the HA-OX group at 7 and 21 days old and the lowest plasma T₃ and T₄ levels and highest PCV values were found in the LA-NOX group at 42 days old. However, some researchers have suggested that there is not always an association between ascites syndrome and the haematocrit values (Shlosberg et al., 1998; Hassanzadeh et al., 2000; Scheele et al., 2005). As reported by Zhang et al. (2007), significant increase in the haematocrit and Hb values were obtained in the birds reared at high altitude compared with those birds reared at low altitude. In contrast to our results, Bahadoran et al. (2010) found that low-altitude incubated chickens showed higher haematocrit values than high altitude incubated chickens only at 7 days old, and the plasma T₃ and T₄ levels were not affected by the two altitudes during the growing period. This may be attributed to the chronic hypobaric hypoxia, which results in a pronounced increase in O_2 capacity and the hematocrit ratio; hence, the Hb values were significantly increased in high-altitude pigeons (Maginniss et al., 1997).

Results reported here were similar to the findings of Hassanzadeh *et al.* (2004), who found that chicks hatched at high altitude, then grown at low altitude, showed significantly higher plasma T_4 concentrations than high-altitude grown birds. Özkan *et al.* (2006) found that ascitic birds had significantly higher hematocrit values than healthy birds and the plasma T_3 concentration was significantly lower in ascitic broilers than in healthy ones at 30 and 37 days old. The low O_2 pressure in high-altitude incubators is an important factor that can alter the development of prenatal and postnatal chicks (Decuypere, 2002; Hassanzadeh, 2009). At high altitudes, the low partial pressure of O_2 causes hypoxia with lower lung activity and higher arterial pressure and affects growth rates (Esmail, 2012).

Conclusions

The hypoxic conditions during the hatching period affected several blood parameters, such as plasma T_3 , plasma T_3/T_4 and Hb levels, in one-day-old chicks. At high altitude, the supplemental O_2 during the hatching period improved chick quality, but did not improve the subsequent FCR and feed consumption performance of chickens when they were reared at low altitude. Better growth and feed consumption performance was observed for the broiler chicks that were hatched and reared at low altitude. The results of the current study add to the broiler producers' knowledge base and contribute to the scientific literature, and may help to clarify the effects of high altitude and O_2 supplementation during hatching on the growth performance of broiler chicks, because many broiler producers obtain their chicks from high-altitude hatcheries and rear them at many altitudes.

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