The effects of diet supplemented with sodium bicarbonate upon blood pH, blood gases and eggshell quality in laying geese

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ABSTRACT: The effects of diet supplemented with sodium bicarbonate ($NaHCO_3$) upon blood pH, blood gases and eggshell quality during the laying cycle in geese were investigated. Fourteen geese aged 2 yr old were divided into two groups as; control (Group C, $n = 7$) and 0.5% $NaHCO_3$-supplemented group (Group T, $n = 7$). After 15 days of adaptation period, blood samples were collected every 6 h during a single laying cycle (over 42 h) and the data obtained were analysed for the pH, base excess (BE-B), HCO_3^- concentration, partial CO_2 pressure (pCO_2) and total CO_2 concentration (tCO_2). The parameters of eggshell quality (i.e. thickness and weight) were also measured following the laying. No correlation was found between the groups for the same blood parameters measured. But, there was a significant correlation (min. $r = 0.946$ and $P < 0.05$) between all the parameters except for the pH in the groups. Following $NaHCO_3$ supplementation of diet however, there was no significant improvement in eggshell thickness and weight. These findings indicate that the $NaHCO_3$ supplementation of diet may support the maintenance of venous blood pH, BE-B, HCO_3^-, pCO_2 and tCO_2 levels at the physiological ranges which are required for normal health and production status of goose during the laying cycle.

Keywords: feeding; bicarbonate; blood; laying period; goose

Poultry are very sensitive animals to blood acid-base disorders (Ergun, 1992) and thus, the blood pH should be close to physiological limits of 7.35 to 7.45 (Dibartola, 1992; Carlson, 1997). This is necessary for the maintenance of protein structure and function, which is an essential condition for normal progression of metabolic events. A deviation from these physiological ranges may cause predisposition to many microbiological diseases, metabolic disorders and losses of productivity, etc. (Haskins, 1977; Dibartola, 1992; Carlson, 1997). This is also the case in geese, as with other animals (Ronald and George, 1988). Blood pH is closely related to some other parameters which can be described with an equation of

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

$$\Uparrow$$

Carbonic Anhydrase

Hence, the changes in any parameter can affect others (Dibartola, 1992; Carlson, 1997). The eggshell is rich in $CaCO_3$. The necessary Ca^{++} is provided form the bone or nutrients, while CO_3^- is obtained from blood HCO_3^-. Free hydrogen ions decrease the blood pH and thus influence the parameters given above. In hens, both blood pH and HCO_3^- levels decline to their minimum levels at 22nd h during the laying cycle (Ergun, 1992). The changes in CO_2 levels can affect both the blood pH and eggshell quality (Card and Nesheim, 1972; Hughes, 1988). Likewise, the blood acidity affects the eggshell formation and *vice versa* (Card and Nesheim, 1972; Dikicioglu, 1990; Ergun, 1992). Additionally, Wideman and Buss (1985) reported that some hens with thin eggshell had markedly lower blood pH and HCO_3^- than in those having thicker shells.

The eggshell quality is a crucial factor in the production of eggs. Since the formation of eggshell takes a long time in geese, they generally lay in every other day (Tilki and Inal, 2004). In the literature, a considerable number of studies aimed at improving the eggshell quality. To this, a number of workers used a variety of diets supplemented with sodium bicarbonate ($NaHCO_3$). A percentage of zero to 1.5 $NaHCO_3$ was reported to be ineffective (Choi and Han, 1983; Grizzle et al., 1992) while 0.3–2% increased the eggshell quality (Markled and El-Gammal, 1977; Davison and Wideman, 1992; Balnave and Muheereza, 1997). Of these however, Davison and Wideman (1992) noted in hens that 3% $NaHCO_3$ led to eggs without shells.

A number of workers reported that $NaHCO_3$ (as supplementation or infusion) increased the blood pH (Junqueira et al., 1984; Bottje and Harrison, 1985; Glahn et al., 1988; Squires and Julian, 2001). Of these, the former workers also reported that the blood base excess (BE-B), HCO_3^- and CO_2 levels were markedly increased following the $NaHCO_3$ supplementation.

Therefore, the aim of this study was to investigate the blood pH, BE-B, HCO_3^-, CO_2 levels and eggshell quality in geese fed 0.5% $NaHCO_3$ -supplemented diet.

MATERIAL AND METHODS

Animals

Fourteen geese (2 yr old all) in laying period (the whole laying season) were used in winter season (from 15th February to 15th March) in this study. Following the observation of a couple of eggs laid by each individual animal during the laying period, a randomly chosen single laying cycle (i.e. the interval between the two consecutive layings) was considered for the experiment. Our preliminary observations showed that a single laying cycle of local native geese of Kars region (43° E, 40.5° N) varied from 38 to 52 h and each goose laid an average of 10 to 15 eggs within the laying period. However, the geese we used in the present study had the laying cycle of 42 h only. The geese were divided into two groups according to their body weights (bw) as; control group, Group C (n = 7, the average bw = 4.26 kg) and $NaHCO_3$ -supplemented group, Group T (n = 7, the average bw = 4.27 kg).

Feeding

The feed of groups as 'basal diet' were formulated to meet the nutritional requirements of the animals (NRC, 1994). The composition of diets are given in Table 1. The geese in group C were fed with a basal diet while it was supplemented with 0.5% $NaHCO_3$ in Group T. Animals were kept individually in wire cages with a self-feeder (0.5 kg/day/goose) and water *ad libitum* throughout the study, namely

Table 1. Ingredients and chemical composition of different diets given to laying geese

Ingredients (%)	Group C	Group T	Chemical analyses (DM basis)	Group C	Group T
Barley	16	16	Dry matter	90.32	90.36
Corn	45	45	Crude protein	16.79	16.80
Sunflower meal	31	30.5	Crude fibre	6.43	6.36
Limestone	5.5	5.5	Ether extract	4.35	4.33
Vegetable oil	1.5	1.5	Organic matter	87.64	87.28
Dicalcium phosphate, DCP	0.5	0.5	Nitrogen free extract	60.13	59.79
Salt	0.25	0.25	Calcium[b]	2.49	2.49
Vitamin-mineral premix[a]	0.25	0.25	Phosphorus[b]	0.66	0.69
$NaHCO_3$	–	0.5			
Metabolisable Energy (kcal/kg[b])	2910.4	2897.7			

[a]For per kg; vitamin A 4 800 000 IU, vitamin D_3 96 000 IU, vitamin E 12 000 mg, vitamin K_3 1 000 mg, vitamin B_1 1 200 mg, vitamin B_2 2 800 mg, vitamin B_6 1 600 mg, vitamin B_{12}, 6 mg, nicotinamide 16 000 mg, calcium-D-pantothenate 3 200 mg, folic acid 400 mg, D-biotin 18 mg, vitamin C 20 000 mg, chlorine 50 000 mg, manganese 32 000 mg, iron 16 000 mg, zinc 24 000 mg, copper 2 000 mg, iodine 160 mg, cobalt 40 mg, selenium 60 mg, antioxidant 4 000 mg
[b]calculated from the tabular values (NRC, 1994)

starting from 15 days prior to (for adaptation to all the experimental conditions) and until the end of experiment. The environmental temperature where the animals were kept was at around 15°C and a continuous (24 h) lighting schedule was applied.

Blood collections

The geese were monitored for the presence of a laid egg during each consecutive hour. Once an egg was laid, the first blood sample was taken and this was presumed as the first (one h) sample. From this time onwards, the collections were repeated every 6 h during the whole laying cycle. Since the next egg was seen just before (or around) the 42th, no further sample was collected after 36 hours.

Blood samples (one ml) were collected from the vein, *v. cutanea ulnaris* (in plastic syringes containing 500 IU/ml heparin). Following the collections, anaerobic condition of samples was obtained by closing the syringes with a glass putty and immediately transferred to the laboratory at zero to 5°C. The analyses were made within 30 min of collection (Haskins, 1977; Ashwood, 1994; Beaulieu et al., 1999).

Laboratory Analyses

Chemical analysis. The parameters of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) of diets were determined by AOAC (1990). The values of metabolisable energy (ME), calcium (Ca) and phosphorus (P) were also calculated from the tabular values (NRC, 1994).

Analysis of blood samples. The parameters of blood pH, base excess of blood (BE-B, mmol/l), HCO_3^- concentration (HCO_3^-, mmol/l), partial CO_2 pressure (pCO_2, mmHg) and total CO_2 concentration (tCO_2, mmol/l) were analysed by a Rapid Lab 248 pH/Blood Gas Analyser (Chiron Diagnostics, USA).

Eggshell quality measurements. Following demembranisation and drying (in a drying oven at 50°C for 48 h), the eggshell weight was measured by an analytical scale (sensitive at 0.0001 g) while the thickness was measured by a calliper compass (sensitive at 0.01 mm).

Statistical analyses

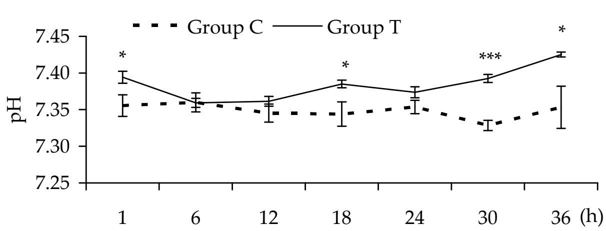
Data (represented as mean ± SEM) from the parameters of blood and of eggshell quality of geese during the laying cycle were analysed by one way analysis of variance (ANOVA) using Minitab statistical software programme (Minitab, 1998). Pearson's correlation test was also used to analyse the interrelationships of each blood parameters during the cycle. Differences of the data between the groups were considered significant when $P < 0.05$.

RESULTS

The values of blood pH, BE-B, HCO_3^-, pCO_2 and tCO_2 of geese fed by different diets during the laying cycle are given in Figures 1–5.

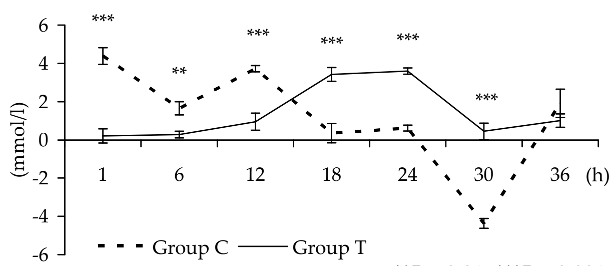
No significant correlation was found between the same blood parameters of the two groups. However, there was a significant correlation (min. $r = 0.946$ and $P < 0.05$) between the parameters of BE-B, HCO_3^-, pCO_2 and tCO_2 in the groups (Group C: min. $r = 0.998$ and $P = 0.001$; Group T: min. $r = 0.946$ and $P = 0.001$).

Although there was a numerical increase of the values of both eggshell thickness and weight in Group T (compared to controls), but differences within each of the parameters were not significant



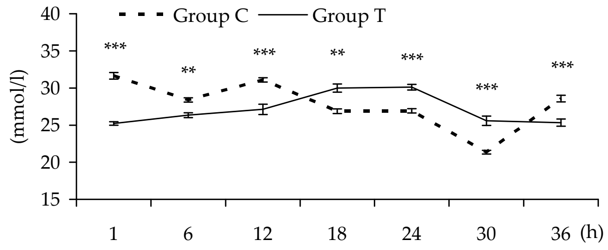
*$P < 0.05$, ***$P < 0.001$

Figure 1. Venous blood pH of geese in different feeding groups during the laying cycle



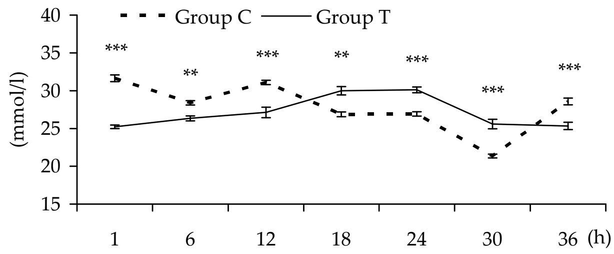
**$P < 0.01$, ***$P < 0.001$

Figure 2. Venous BE-B of geese in different feeding groups during the laying cycle



**P < 0.01, ***P < 0.001

Figure 3. Venous blood HCO_3^- of geese in different feeding groups during the laying cycle



**P < 0.01, ***P < 0.001

Figure 4. Venous blood pCO_2 of geese in different feeding groups during the laying cycle

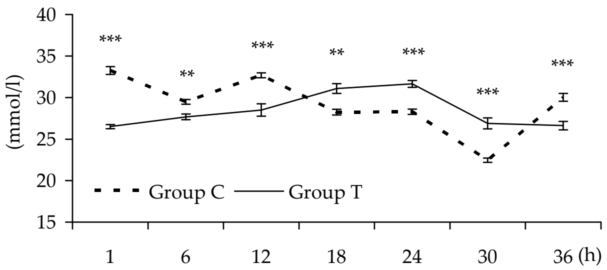


Figure 5. Venous blood tCO_2 of geese in different feeding groups during the laying cycle

**P < 0.01, ***P < 0.001

between the groups. The values of eggshell thickness and weight are given in Table 2.

DISCUSSION

In this paper, we describe the effects of $NaHCO_3$ supplementation on the venous blood pH, BE-B, HCO_3^-, pCO_2 and tCO_2 values and eggshell quality parameters during the laying cycle of geese.

In the present study, $NaHCO_3$-supplemented diet during the laying cycle affected the parameters of blood in treatment group as compared to controls. None of the parameters studied between the groups was correlated with each other. However, the parameters of BE-B, HCO_3^-, pCO_2 and tCO_2 in the groups were significantly correlated together (min. $r = 0.946$; $P = 0.001$).

In laying hens, the blood pH dramatically decrease particularly at the 22nd h of the cycle (Card and Nesheim, 1972). In control group of the present study, the corresponding time was the 30th. This situation may lead, at least to some extent, to a predisposition to possible microbiological infections and metabolic disorders (Haskins, 1977; Dibartola, 1992; Carlson, 1997). However, the potential debilitating effects of low levels of pH might be overcome by using $NaHCO_3$ supplementation in the diet. Indeed, it was reported that $NaHCO_3$-supplemented diet causes an increased blood pH of broiler chickens (Squires and Julian, 2001) and of laying hens (Junqueira et al., 1984). Similar observations were made in the present study such that the supplementation provided the stability of blood pH to remain at physiological limits (around 7.4), as compared to controls. This was also the case in the previous studies (Ronald and George, 1988; Dibartola, 1992; Carlson, 1997).

The base excess in blood (BE-B) is an important parameter related to acid-base status and its level has to be at a level of 0 ± 4 mmol/l for compensation of acid-base fluctuations (Haskins, 1977).

Table 2. Eggshell quality (thickness and weight) of geese in different feeding groups during the laying cycle (mean ± SEM)

Eggshell quality	Group C	Group T	Significance
Thickness (mm)	0.55 ± 0.030	0.61 ± 0.002	NS
Weight (g)	13.23 ± 0.82	14.61 ± 0.47	NS

NS = not significant

A substantial deviation from this concentration inevitably affects the blood pH and thus other relevant physiological and biochemical functions of the body (Carlson, 1997). Junqueria et al. (1984) reported that $NaHCO_3$ supplementation markedly increased the base excess in laying hens. The present findings showed that the supplementation sustains the levels around optimal during the laying cycle.

The blood HCO_3^- level is also a crucial part of acid-base (and of BE-B) balance system in the body. During the laying cycle, the HCO_3^- is used for CO_3^- production of the eggshell and its concentration declines, by the time, as it is used for this process. It was previously shown that the HCO_3^- concentration declines to its minimum levels at the 22nd h of the laying cycle of hens (Dikicioglu, 1990; Ergun, 1992). In the present study however, this was observed at a later time (approximately the 30th h) in geese. This means that the required HCO_3^- in the blood was obtained from $NaHCO_3$ in the diet. In laying hens, similar results were also reported previously (Junqueira et al., 1984; Bottje and Harrison, 1985; Glahn et al., 1988; Squires and Julion, 2001).

Additionally, the present findings showed that the parameters of pCO_2 and tCO_2 in each group were significantly correlated together (r = 1.000; P = 0.000). A significant correlation was also found between those of CO_2, HCO_3^- and BE-B within their own groups (min. r = 0.946; P = 0.001). These findings are in parallel with the literature (Dibartola, 1992; Carlson, 1997). Furthermore, the levels of blood CO_2 increased following the $NaHCO_3$ supplementation in the present study, as reported previously (Junqueira et al., 1984).

There have been some reports of the effects of $NaHCO_3$ supplementation improving the eggshell thickness (Markled and El-Gammal, 1977; Choi and Han, 1983; Davison and Wideman, 1992; Grizzle et al., 1992; Balnave and Muheereza, 1997; Hayat et al., 1999). However, no such significant improvement was observed in either the eggshell thickness or weight following 0.5% $NaHCO_3$ supplementation of diet in laying geese in the present study. Nevertheless, it is likely that a higher percentage of $NaHCO_3$ supplementation might lead to a superior eggshell quality.

In conclusion, the present study suggests that the $NaHCO_3$ supplementation of diet may support the maintenance of venous blood pH, BE-B, HCO_3^-, pCO_2 and tCO_2 levels at the physiological ranges which are required for normal health and production status of goose during the laying cycle.

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