

Plant Based High Energy Mash Diets Supplemented With Nahco₃, L-arginine+ Vitamin-c and Vegetable Oils Are Effective Against Tachycardia and Polycythemia in Broiler Chicken

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Abstract

The study aimed to investigate if plant based high energy mash diets supplemented with NaHCO₃, L-arginine + vitamin-C and vegetable oils were effective against tachycardia and polycythemia in the commercial broiler chicken. Total 256 Ross-308 day old male broiler chicks were randomly distributed into eight dietary treatment groups in a three way 2³ factorial arrangements (Three factors, i.e., NaHCO₃, L-arginine + vitamin-C and vegetable oil each with two levels, e.g., 0 and 0.1% for NaHCO₃ and L-arginine + vitamin-C; 3 and 4% of vegetable oil supplemented with basal diet). Iso-caloric and iso-nitrogenous diets were formulated and supplied *ad libitum*. The average daily feed intake (ADFI), average daily gain (ADG), feed efficiency (FE), carcass characteristics, cardio-pulmonary morphometry, total protein (TP), hemoglobin (Hb), triiodothyronine (T₃), incidence of tachycardia and polycythemia were examined up to 35 d. Supplementation of NaHCO₃ decreased (p<0.001) the ADFI at 1-14 d, 15-35 d, 1-35 d, improved (p<0.01) the FE at 1-14 d and increased (p<0.05) the serum TP. Dietary L-arginine + vitamin-C decreased (p<0.01) the heart rate without affecting the performance parameters, carcass characteristics and hemato-biochemical indices. Supplementation of vegetable oil decreased (p<0.01) the ADFI at 1-14d, 15-35 d, 1-35 d, increased (p<0.01) the ADG at 1-14d, improved (p<0.001) the FE at 1-14 d and increased (p<0.05) the heart rate, Hb and PCV. Further, NaHCO₃, L-arginine + vitamin-C and vegetable oil synergistically interacted to decrease the left and right ventricular weight, RV:TV and increased the T₃ without affecting overall performance, carcass characteristics and hemato-biochemical indices. It was concluded that, plant based high energy mash diets are not susceptible to tachycardia and polycythemia. Addition of NaHCO₃, L-arginine + vitamin-C ameliorate the propensity of tachycardia and polycythemia without deteriorating performance, carcass characteristics and hemato-biochemical indices of the commercial broiler birds in a dose dependent manner.

Introduction

Tachycardia in broiler is a patho-physiological interplay between the lungs and the heart initiated by the terminal consequences of the excessively elevated blood pressure within the pulmonary circulation (Wideman et al. 2013; Khajali and Wideman 2016). A broiler chick initially weighing around 40 g at hatch is likely to attain more than 4000 g in 8 weeks. This elevated growth of almost 100-fold in just 8 weeks cannot be sustained devoid of similar remarkable increases in the functional capability of the heart and lungs (Wideman et al., 2013). The cardiac output increases 100 fold in 8 weeks post hatch, ranging from 8ml/min for a 40 g chick to around 800 ml/min for a broiler weighing 4 kg (Wideman 1999; Wideman et al. 2013). Furthermore, in the study of Decuyper et al. (2000) on the muscle fibre typology it was evident that the choice for increased breast meat yield did not result in a proportionate increase in the heart, blood and lung weight due to the relative independence of glycolytic white muscle to the requirement for their oxygen. Due to these discrepancies, pulmonary vascular capacity in broiler birds is restricted and only a bit able to cope with continuous increases in the cardiac yield (Wideman and Bottje, 1993; Wideman, 2000). Therefore, modern broiler birds are prone to initially tachycardia and later on to the progressive development of pulmonary hypertension syndrome (PHS).

The PHS stimulates the red blood cells racing too rapidly through the pulmonary vasculature. Thus, the RBCs cannot achieve full blood-gas equilibrium because of a short transit time at the gas exchange surfaces leading to incomplete diffusive exchange of O₂ and CO₂ (Henry and Fedde, 1970; Powell et al., 1985). This inadequate residence time, thus, causes blood exiting the lungs and to enter the systemic flow with a lower than the usual partial pressure of O₂ leading to hypoxemia. In the systemic flow, hypoxemia evokes extensive arteriolar dilatation to raise the blood flow and reinstate ample O₂ delivery to the organs as well as tissues (Wideman et al. 1996, 1997, 2000; Wideman 2000; Wideman and Tackett 2000; Ruiz-Feria and Wideman 2001). The systemic arteriolar vasodilatation lets blood to way out to the large arteries more quickly accompanied by increment in the rate at which venous blood comes back to the right ventricle. The rise in venous return and the commencement of systemic arteriolar hypotension automatically stimulate the heart to increase the cardiac output leading to tachycardia. Additionally, persistent hypoxemia stimulates hematopoietin and markedly increases the hematocrit (Burton et al., 1971; Julien et al., 1985).

Increased hematocrit results polycythemia which boosts up blood thickness and is the most important reasons of the increased resistance to the blood-flow that consequences in PHS (Snyder, 1971; Penney et al., 1988). High energy pellet diets provoke PHS by stimulating lipolysis and being deficient in protein and thereby arginine as well. Plant proteins, by virtue, are more susceptible

to be deficient in critical amino acids, i.e., lysine, methionine and arginine. Consequently, we warned that plant based high energy mash diet may prone to reduce the performance of the birds. We therefore, aimed to justify if plant based high energy mash diet supplemented with L-arginine + vitamin-C and sodium bicarbonate was effective against tachycardia and polycythemia in commercial broiler birds.

Materials And Methods

Study design, animals and housing

The experiment was conducted in a three way 2³ factorial arrangements (Three dietary factors, i.e., NaHCO₃, L-arginine + vitamin-C and vegetable oil each with two levels, e.g., NaHCO₃, 0 and 0.1%; L-arginine + vitamin-C, 0 and 0.1%; Vegetable oil, 3 and 4% of the basal diet). Total 256 Ross-308 day old male broiler chicks were randomly distributed into eight dietary treatment groups with four replicates having 8 birds per pen (Table 1). The chicks were purchased from Nahar Agro Ltd., Chattogram, Bangladesh. All chicks were examined for male, grade A, uniform size without abnormalities. Floor space for each bird was 0.17 square feet in brooding box and 1 square feet in the cage. The birds were exposed to continuous lighting. The chicks were brooded at a temperature of 95°F, 90°F, 85°F and 80°F for the 1st, 2nd, 3rd and 4th weeks, respectively with the help of incandescent bulbs. Room temperature and humidity were measured by using wall mounted indoor analog thermo-hygrometer. Before arrival of the chicks, the shed was thoroughly cleaned and washed by using tap water with caustic soda. For disinfection, phenyl solution (1% v/v) was sprayed on the floor, corners and ceiling. Following spray, cleaning was done by using brush and clean water. Brooding boxes, rearing cages and pens were cleaned in the same manner. After cleaning and disinfection, the house was left empty one week for proper drying. After drying, all doors and windows was closed. The room was fumigated with single strength fumigant (Adding 40 ml formalin to 20 g KMnO₄ for 100 cubic feet area) and sealed for 24 hours. On the next day, lime was spread on the floor and around the shed. Footbath containing potassium permanganate (1% w/v) was kept at the entrance of the poultry shed and changed daily. Feeders and drinkers were cleaned and washed with Timsen® solution (0.3% v/v) daily. All birds were vaccinated against Newcastle and Gumboro disease with both the primary and booster doses.

Table 1
Design of the experiment

Dietary treatments			No. of treatments	No. of replicates	No. of birds
NaHCO ₃ (%)	L-arginine + vitamin-C (%)	Vegetable oil (%)			
0	0	3	1	4	4 × 8 = 32
0	0	4	1	4	4 × 8 = 32
0	0.10	3	1	4	4 × 8 = 32
0	0.10	4	1	4	4 × 8 = 32
0.10	0	3	1	4	4 × 8 = 32
0.10	0	4	1	4	4 × 8 = 32
0.10	0.10	3	1	4	4 × 8 = 32
0.10	0.10	4	1	4	4 × 8 = 32
Total	2	2	8	32	8 × 4 × 8 = 256

Experimental diets

Feed ingredients were purchased from local market. During purchase, wholesomeness and date of expiry was checked. Dry mash feed was provided to the birds throughout the whole experimental period. Eight different types of starter (1–14 d) and finisher (15–35 d) diets (Table 2–3) were formulated and supplied to the birds. All rations were iso-caloric and iso-nitrogenous. Feed was

prepared manually and supplied ad-libitum to the birds on round small feeder for 1–10 days. After 10th day, small round feeders and waterers were replaced by large linear feeders and bell drinkers.

Table 2
Formulation of the starter diets (1–14 d) for the experimental broiler birds

Amount (g/kg)	Dietary treatments ¹							
	N ₀ A ₀ V ₃	N ₀ A ₁ V ₃	N ₁ A ₀ V ₃	N ₁ A ₁ V ₃	N ₀ A ₀ V ₄	N ₀ A ₁ V ₄	N ₁ A ₀ V ₄	N ₁ A ₁ V ₄
Maize	57.75	57.75	57.75	57.75	56.58	56.58	56.58	56.58
Rice polish	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Soybean oil	1.50	1.50	1.50	1.50	2.00	2.00	2.00	2.00
Soybean meal	37.00	37.00	37.00	37.00	37.30	37.30	37.30	37.30
Limestone	1.48	1.38	1.38	1.28	1.72	1.62	1.62	1.52
DCP	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
L-Lysine	0.12	0.12	0.12	0.12	0.20	0.20	0.20	0.20
DL-Methionine	0.20	0.20	0.20	0.20	0.25	0.25	0.25	0.25
Vitamin premix ²	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
NaHCO ₃	0.00	0.00	0.10	0.10	0.00	0.00	0.10	0.10
L-arginine ³	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05
Vitamin-C ⁴	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05
Feedzyme ⁵	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Emulsifier ⁶	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated values⁷								
Metabolizable energy (kcal/kg)	3000.74	3000.74	3000.74	3000.74	3004.38	3000.74	3000.74	3000.74
Crude protein	22.00	22.00	22.00	22.00	22.01	22.00	22.00	22.00
Crude fibre	3.42	3.42	3.42	3.42	3.41	3.42	3.42	3.42
Ether extract	3.95	3.95	3.95	3.95	4.42	3.95	3.95	3.95
Calcium	0.92	0.92	0.92	0.92	1.09	0.92	0.92	0.92
Phosphorus	0.65	0.65	0.65	0.65	0.69	0.65	0.65	0.65
Available phosphorus	0.36	0.36	0.36	0.36	0.40	0.36	0.36	0.36
Sodium	0.01	0.01	0.04	0.04	0.01	0.01	0.04	0.04
Potassium	0.91	0.91	0.91	0.91	0.92	0.91	0.91	0.91
Magnesium	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Manganese (mg/kg)	80.23	80.23	80.23	80.23	80.29	80.23	80.23	80.23
Zinc (mg/kg)	86.05	86.05	86.05	86.05	85.92	86.05	86.05	86.05
Copper (mg/kg)	17.49	17.49	17.49	17.49	17.51	17.49	17.49	17.49
Iron (mg/kg)	156.30	156.30	156.30	156.30	156.36	156.30	156.30	156.30

Amount (g/kg)	Dietary treatments ¹							
	N ₀ A ₀ V ₃	N ₀ A ₁ V ₃	N ₁ A ₀ V ₃	N ₁ A ₁ V ₃	N ₀ A ₀ V ₄	N ₀ A ₁ V ₄	N ₁ A ₀ V ₄	N ₁ A ₁ V ₄
Lysine	1.31	1.31	1.31	1.31	1.39	1.31	1.31	1.31
Leucine	1.88	1.88	1.88	1.88	1.87	1.88	1.88	1.88
Iso-leucine	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Valine	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04
Threonine	1.21	1.21	1.21	1.21	1.22	1.21	1.21	1.21
Methionine	0.54	0.54	0.54	0.54	0.59	0.54	0.54	0.54
Tryptophan	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Phenylalanine	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Cystine + methionine	0.73	0.73	0.73	0.73	0.78	0.73	0.73	0.73
Analyzed values (%)⁸								
AGE (kcal/kg) ⁹	3513.96	3506.92	3510.44	3503.40	3496.35	3499.87	3492.83	3489.31
Crude protein	21.56	21.12	20.90	21.34	21.78	21.56	21.12	21.56
Crude fibre	3.35	3.28	3.25	3.32	3.39	3.35	3.28	3.35
Ether extract	3.87	3.79	3.75	3.83	3.91	3.87	3.79	3.87
Calcium	0.90	0.88	0.87	0.89	0.91	0.90	0.88	0.90
Phosphorus	0.64	0.62	0.62	0.63	0.64	0.64	0.62	0.64
¹ N ₀ A ₁ V ₃ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;								
N ₁ A ₀ V ₃ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, vegetable oil 3%;								
N ₁ A ₁ V ₃ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;								
N ₀ A ₀ V ₄ = NaHCO ₃ 0%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;								
N ₀ A ₁ V ₄ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;								
N ₁ A ₀ V ₄ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;								
N ₁ A ₁ V ₄ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;								
² Per 2500 g contained: Beta-Carotene (Vitamin A) 12000000 IU, Cholecalciferol (Vit-D ₃) 2400000 IU, Alpha-Tocopherol (Vit-E) 23 g, Menadione (Vit-K ₃) 2 g, Thiamine (Vit-B ₁) 2.5 g, Riboflavin (Vit-B ₂) 5 g, Pyridoxine (Vit-B ₆) 4 g, Nicotinic acid 40 g, Calcium-D-Pantothenete 12.5 g, Cobalamin (Vit-B ₁₂) 12 mg, Folic acid 800 mg, Biotin (Vit-B ₇) 100 mg, Cobalt 400 mg, Copper 10 g, Iron 60 g, Iodine 400 mg, Manganese 60 g, Zinc 50 g, Selenium 150 mg, DL-Mehionine 100 g, L-Lysine 60 g, Calcium 679.6 g;								
³ L-Arginine 99.0%, Vitamondo private limited, 89/19, Kamarajar colony, Musiri, TrichyTiruchirappalli TN 621211 India;								
⁴ Vitamin-C 97%, Sridhar Enterprises, Ayanambakkam, Chennai, Tamil Nadu, India;								
⁵ Per 100 g contained: Cellulase 20000 IU, Xylanase 200000 IU, Protease 20 IU, Amylase 40000 IU, Phytase 20 IU, Pectinase 1400 IU, Invertase 400 IU, Hemicellulose 500 IU, Lipase 20 IU, α-Galactosidase 100 IU;								

Amount (g/kg)	Dietary treatments ¹							
	N ₀ A ₀ V ₃	N ₀ A ₁ V ₃	N ₁ A ₀ V ₃	N ₁ A ₁ V ₃	N ₀ A ₀ V ₄	N ₀ A ₁ V ₄	N ₁ A ₀ V ₄	N ₁ A ₁ V ₄
⁶ Contained: Phosphatidyl-choline (PC) 16%, Phosphatidyl-ethanolamine (PE) 10%, Phosphatidic-acid (PA) 11%, Phosphatidyl-inositol (PI) 8%, Lyso-phosphatidyl-choline (LPC) 8%, Lyso-phosphatidyl-ethanolamine (LPE) 13%, Lyso-phosphatidic-acid (LPA) 6%, Lyso-phosphatidyl-inositol (LPI) 8%, Carrier 20% (Brand-Molimen, Country of origin-Spain);								
⁷ Unit was considered as % or otherwise stated;								
⁸ As per standard procedure (AOAC, 2019);								
⁹ Apparent gross energy (kcal/kg).								

Table 3
Formulation of the finisher diets (15–35 d) for the experimental broiler birds

Amount (g/kg)	Dietary treatments ¹							
	N ₀ A ₀ V ₃	N ₀ A ₁ V ₃	N ₁ A ₀ V ₃	N ₁ A ₁ V ₃	N ₀ A ₀ V ₄	N ₀ A ₁ V ₄	N ₁ A ₀ V ₄	N ₁ A ₁ V ₄
Maize	60.00	60.00	60.00	60.00	56.00	56.00	56.00	56.00
Rice polish	1.25	1.25	1.25	1.25	2.25	2.25	2.25	2.25
Soybean oil	3.00	3.00	3.00	3.00	4.00	4.00	4.00	4.00
Soybean meal	32.10	32.10	32.10	32.10	32.60	32.60	32.60	32.60
Limestone	1.88	1.78	1.78	1.68	2.00	1.90	1.90	1.80
DCP	0.80	0.80	0.80	0.80	1.95	1.95	1.95	1.95
L-Lysine	0.12	0.12	0.12	0.12	0.25	0.25	0.25	0.25
DL-Methionine	0.20	0.20	0.20	0.20	0.30	0.30	0.30	0.30
Vitamin premix ²	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
NaHCO ₃	0.00	0.00	0.10	0.10	0.00	0.00	0.10	0.10
L-arginine ³	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05
Vitamin-C ⁴	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05
Feedzyme ⁵	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Emulsifier ⁶	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated values⁷								
Metabolizable energy (kcal/kg)	3102.26	3102.26	3102.26	3102.26	3101.38	3101.38	3101.38	3101.38
Crude protein	20.02	20.02	20.02	20.02	20.03	20.03	20.03	20.03
Crude fibre	3.23	3.23	3.23	3.23	3.26	3.26	3.26	3.26
Ether extract	5.49	5.49	5.49	5.49	6.55	6.55	6.55	6.55
Calcium	1.08	1.08	1.08	1.08	1.40	1.40	1.40	1.40
Phosphorus	0.65	0.65	0.65	0.65	0.86	0.86	0.86	0.86
Available phosphorus	0.37	0.37	0.37	0.37	0.57	0.57	0.57	0.57
Sodium	0.01	0.01	0.04	0.04	0.01	0.01	0.04	0.04
Potassium	0.80	0.80	0.80	0.80	0.83	0.83	0.83	0.83
Magnesium	0.12	0.12	0.12	0.12	0.13	0.13	0.13	0.13
Manganese (mg/kg)	79.76	79.76	79.76	79.76	81.89	81.89	81.89	81.89
Zinc (mg/kg)	87.93	87.93	87.93	87.93	93.01	93.01	93.01	93.01
Copper (mg/kg)	16.76	16.76	16.76	16.76	16.84	16.84	16.84	16.84
Iron (mg/kg)	148.05	148.05	148.05	148.05	148.63	148.63	148.63	148.63

Amount (g/kg)	Dietary treatments ¹							
	N ₀ A ₀ V ₃	N ₀ A ₁ V ₃	N ₁ A ₀ V ₃	N ₁ A ₁ V ₃	N ₀ A ₀ V ₄	N ₀ A ₁ V ₄	N ₁ A ₀ V ₄	N ₁ A ₁ V ₄
Lysine	1.18	1.18	1.18	1.18	1.32	1.32	1.32	1.32
Leucine	1.73	1.73	1.73	1.73	1.72	1.72	1.72	1.72
Iso-leucine	0.85	0.85	0.85	0.85	0.86	0.86	0.86	0.86
Valine	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Threonine	1.08	1.08	1.08	1.08	1.09	1.09	1.09	1.09
Methionine	0.52	0.52	0.52	0.52	0.61	0.61	0.61	0.61
Tryptophan	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.25
Phenylalanine	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Cystine + methionine	0.71	0.71	0.71	0.71	0.80	0.80	0.80	0.80
Analyzed values (%)⁸								
AGE (kcal/kg) ⁹	3603.78	3596.56	3602.17	3592.95	3589.72	3592.95	3596.56	3589.33
Crude protein	19.82	19.91	19.90	19.88	20.02	19.82	19.92	19.89
Crude fibre	3.17	3.10	3.07	3.13	3.20	3.17	3.10	3.17
Ether extract	5.38	5.27	5.22	5.33	5.44	5.38	5.27	5.38
Calcium	1.06	1.04	1.03	1.05	1.07	1.06	1.04	1.06
Phosphorus	0.64	0.62	0.62	0.63	0.64	0.64	0.62	0.64
¹ N ₀ A ₁ V ₃ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;								
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N ₁ A ₁ V ₄ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;								
² Per 2500 g contained: Beta-Carotene (Vitamin A) 12000000 IU, Cholecalciferol (Vit-D ₃) 2400000 IU, Alpha-Tocopherol (Vit-E) 23 g, Menadione (Vit-K ₃) 2 g, Thiamine (Vit-B ₁) 2.5 g, Riboflavin (Vit-B ₂) 5 g, Pyridoxine (Vit-B ₆) 4 g, Nicotinic acid 40 g, Calcium-D-Pantothenete 12.5 g, Cobalamin (Vit-B ₁₂) 12 mg, Folic acid 800 mg, Biotin (Vit-B ₇) 100 mg, Cobalt 400 mg, Copper 10 g, Iron 60 g, Iodine 400 mg, Manganese 60 g, Zinc 50 g, Selenium 150 mg, DL-Methionine 100 g, L-Lysine 60 g, Calcium 679.6 g;								
³ L-Arginine 99.0%, Vitamondo private limited, 89/19, Kamarajar colony, Musiri, TrichyTiruchirappalli TN 621211 India;								
⁴ Vitamin-C 97%, Sridhar Enterprises, Ayanambakkam, Chennai, Tamil Nadu, India;								
⁵ Per 100 g contained: Cellulase 20000 IU, Xylanase 200000 IU, Protease 20 IU, Amylase 40000 IU, Phytase 20 IU, Pectinase 1400 IU, Invertase 400 IU, Hemicellulose 500 IU, Lipase 20 IU, α-Galactosidase 100 IU;								

Amount (g/kg)	Dietary treatments ¹							
	N ₀ A ₀ V ₃	N ₀ A ₁ V ₃	N ₁ A ₀ V ₃	N ₁ A ₁ V ₃	N ₀ A ₀ V ₄	N ₀ A ₁ V ₄	N ₁ A ₀ V ₄	N ₁ A ₁ V ₄
⁶ Contained: Phosphatidyl-choline (PC) 16%, Phosphatidyl-ethanolamine (PE) 10%, Phosphatidic-acid (PA) 11%, Phosphatidyl-inositol (PI) 8%, Lyso-phosphatidyl-choline (LPC) 8%, Lyso-phosphatidyl-ethanolamine (LPE) 13%, Lyso-phosphatidic-acid (LPA) 6%, Lyso-phosphatidyl-inositol (LPI) 8%, Carrier 20% (Brand-Molimen, Country of origin-Spain);								
⁷ Unit was considered as % or otherwise stated;								
⁸ As per standard procedure (AOAC, 2019);								
⁹ Apparent gross energy (kcal/kg).								

Chemical analysis

Chemical analyses of the experimental diets were carried out in triplicate for dry matter (DM), crude protein (CP), crude fiber (CF), nitrogen free extracts (NFE), ether extracts (EE) and total ash (TA) in the animal nutrition postgraduate laboratory, Chattogram Veterinary and Animal Sciences University, Chattogram as per standard procedure (AOAC, 2019). Moisture was estimated by Hot air oven (SLN-115, Pol-Eko-Aparatus SP.J, Poland). CP was estimated by micro Kjeldhal apparatus (Kjeldhal digestion unit SBS800, Kjeldhal distillation unit D1000, FoodAlyt, Germany). CF was estimated by using Ankom Fiber Analyzer (Fiberbag System-6, Gerhardt, Germany). EE was estimated by using Soxtec (RS-232, SER-148, VelpScientifica, 155 Keyland Court, Bohemia, NY 11716 - US). TA was estimated by the muffle furnace (HYSC, Non Yong Scientific Equipment Company Ltd., 874-1 Wolgye 4-dong, Nowon-gu, Seoul, Korea). Apparent gross energy (AGE) of mixed diets was estimated by using the bomb calorimeter (Parr 6200 Calorimeter, Parr Instruments Co., USA).

Performance parameter

Mortality was recorded as occurred, while average daily feed intake (ADFI), average daily gain (ADG), feed efficiency (FE) were recorded fortnightly. Carcass characteristics, hematological and biochemical parameters were recorded at 5th week. Weight gain was calculated by deducting initial body weight from the final body weight of the birds. Feed intake was calculated by deducting leftover from the total feeds supplied to the birds. The FE was calculated dividing feed intake by weight gain.

Carcass characteristics

At day 35 two birds from each replicate were randomly selected and killed by severing the jugular vein and carotid artery. Once a bird was adequately bled out, it was scalded and defeathered. After defeathering, the birds were eviscerated and the head and feet were removed as per standard technique (Jones, 1984). During evisceration process, abdominal fat, lung, liver, kidney, spleen, gizzard and proventriculus were excised separately and weighed. Dressed birds were weighed to obtain a dressed carcass weight.

Cardio-pulmonary morphometry

The heart was isolated from the carcass immediate after slaughter. The data of heart weight, right ventricular weight, left ventricular weight, right ventricular diameter, left ventricular diameter, right ventricular thickness and left ventricular thickness were measured thereafter by using slide caliper (WiikaVernier Caliper, 150 mm, WA-VC1150) and digital screw gauge (Mitutoyo, Quickmini, Mitutoyo corporation, Japan). The diameter of left and right ventricles were measured perpendicular to the long axis, at the maximum measureable dimensions and mean values were calculated.

Hemato-biochemical test

Samples were analyzed in three different research laboratories, i.e., Postgraduate laboratory of Dairy and Poultry Science, Animal science and Nutrition laboratory and Physiology, Pharmacology and Biochemistry laboratory of CVASU. Blood samples were collected from the carotid artery of three birds from each replicate using 4 ml sterile vacutainer tubes containing anticoagulant (EDTA) for serum tests and without anticoagulant for hematology. The hemoglobin concentration was estimated by hematology analyzer (Celltac Alpha, Nihon Kohden, MEK 6550). Packed cell volume was measured after centrifugation of a small amount of

blood using micro-hematocrit capillary tubes. For serum analysis, clotted blood in the vacutainer tube was centrifuged at 3000 rpm for 20 minutes by the multipurpose centrifuge (DLAB, DMO636, Berkeley, CA 94720 – 3030) and prepared serum was collected into the eppendorf tube by micropipette. Sera samples were marked and stored in -20°C until being analyzed. All biochemical tests were conducted by a hemato-biochemical analyzer (HumaLyzer 3000, Human, Germany). Manufacturer's recommended standard test kits were used for testing serum glucose (Method: GOD-PAP, Ref. 10260, Liquicolor, Human, Germany); serum glutamic oxaloacetic transaminase, SGOT (Method: ALAT IFCC, Ref. 12021, Human, Germany), serum glutamate-pyruvate transaminase, SGPT (Method: ALAT IFCC, Ref. 12022, Human, Germany), albumin (Ref. 10560, Human, Germany); total protein (Ref. 10570, Human, Germany), total cholesterol (Ref. CH 200, Randox, Germany) and T₃ (Bioscience medical, Madrid, Spain).

Statistical analysis

Data were compiled in MS Excel. Raw data related to weight gain, feed intake, FCR, carcass characteristics, cardio-pulmonary morphometry, hematological and biochemical parameters were tested for outliers and multicollinearity by inter quartile range test and variance inflation factors. Normality of variable was checked by using normal probability plot and equality of variances in the response variable was checked by Shapiro Wilk test. Data were analyzed in generalized linear model (GLM) by using Stata 14.1 SE (StataCorp LP, College Station, Texas, USA). Means showing significant differences were compared by Duncan's New Multiple Range Test (Duncan, 1955). Statistical significance was accepted at $p < 0.05$ for Fisher's F-tests. The following statistical model was used:

Y_{ijk}	=	$\mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijkn}$
Where,		
μ	=	The intercept of the regression model;
α_i	=	The effect of the 'i th ' level of the factor 'α' (NaHCO ₃) on the value observed in Y _{ijk} (i = 0, 0.10% of NaHCO ₃ of the basal diet);
β_j	=	The effect of the 'j th ' level of the factor 'β' (Additive) on the value observed in Y _{ijk} (j = 0, 0.10% of L-arginine + vitamin-C of the basal diet);
γ_k	=	The effect of the 'k th ' level of the factor 'γ' (Vegetable oil) on the value observed in Y _{ijk} (k = 3, 4% of vegetable oil of the basal diet);
$(\alpha\beta\gamma)_{ijk}$	=	The interaction effect of the of the 'i th ' level of the factor 'α', the 'j th ' level of the factor 'β', and 'k th ' level of the factor 'γ';
Y _{ijk}	=	The observed value of the variable under study for the 'n th ' repetition of the combination of the 'i th ' level of factor 'α', the 'j th ' level of the factor 'β', and the 'k th ' level of the factor 'γ';
ϵ_{ijk}	=	The random sampling error due to 'i th ' level of the factor 'α', 'j th ' level of the factor 'β', and 'k th ' level of the factor 'γ'.

Results

Performance

Supplementation of NaHCO₃ decreased ($p < 0.001$) ADFI at 1–14 d, 15–35 d, 1–35 d and improved ($p < 0.01$) FE from 0.91 to 0.86 at 1–14 d, although, ADG remained unchanged ($p > 0.05$). Dietary L-arginine + vitamin-C did not influence ($p > 0.05$) the performance parameter of the birds throughout the trial period. Vegetable oil decreased ($p < 0.01$) ADFI at 1-14d, 15–35 d, 1–35 d, increased ($p < 0.01$) ADG at 1-14d and improved ($p < 0.001$) FE at 1–14 d. There were no interactions of NaHCO₃ × L-arginine + vitamin-C × Vegetable oil on performance parameters of the experimental birds (Table 4).

Table 4

Initial live weight (ILW, g/bird/d), final live weight (FLW, g/bird/d), average daily feed intake (ADFI, g/bird/d), average daily gain (ADG, g/bird/d) and feed efficiency (FE, ADFI/ADG) of the broiler birds fed diets supplemented with various levels of NaHCO₃, L-arginine + vitamin-C and vegetable oils

Treatment factors ¹	Performance of broiler birds										
	ILW (g/bird/d)	FLW (g/bird/d)	ADFI (g/bird/d)			ADG (g/bird/d)			FE		
	15–35 d	1–35 d	1–14 d	15–35 d	1–35 d	1–14 d	15–35 d	1–35 d			
NaHCO ₃ (N)											
0.00%	40.97	2109.91	18.54 ^a	115.08 ^a	76.47 ^a	20.36	74.08	52.59	0.91 ^a	1.54	1.29
0.10%	42.73	2187.86	17.56 ^b	113.75 ^b	75.28 ^b	20.33	73.25	52.08	0.86 ^b	1.55	1.27
L-arginine + vitamin-C (A)											
0.00%	41.48	2123.89	18.00	114.33	75.80	20.48	72.52	51.71	0.88	1.56	1.29
0.10%	42.22	2173.87	18.10	114.50	75.94	20.21	74.81	52.97	0.89	1.53	1.27
Vegetable oil (V)											
3.00%	42.13	2120.50	18.48 ^a	115.00 ^a	76.39 ^a	19.93 ^a	74.75	52.82	0.92 ^a	1.54	1.29
4.00%	41.57	2177.26	17.63 ^b	113.83 ^b	75.35 ^b	20.76 ^b	72.58	51.85	0.85 ^b	1.55	1.27
N×A×V											
N ₀ ×A ₀ ×V ₃	41.00	2048.91	18.75	115.33	76.70	20.02	74.54	52.73	0.93	1.54	1.30
N ₀ ×A ₁ ×V ₃	40.73	2129.29	18.00	114.33	75.80	20.88	72.23	51.69	0.86	1.55	1.28
N ₀ ×A ₀ ×V ₄	40.00	2104.76	19.42	116.33	77.57	20.19	75.88	53.61	0.96	1.52	1.30
N ₀ ×A ₁ ×V ₄	42.13	2156.67	18.00	114.33	75.80	20.35	73.67	52.34	0.88	1.53	1.27
N ₁ ×A ₀ ×V ₃	42.75	2160.71	18.00	114.33	75.80	20.05	72.66	51.62	0.89	1.57	1.30

¹N₀A₁V₃ = NaHCO₃0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;

N₁A₀V₃ = NaHCO₃0.1%, L-arginine 0% + Vitamin-C 0%, vegetable oil 3%;

N₁A₁V₃ = NaHCO₃0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;

N₀A₀V₄ = NaHCO₃ 0%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;

N₀A₁V₄ = NaHCO₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;

N₁A₀V₄ = NaHCO₃0.1%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;

N₁A₁V₄ = NaHCO₃0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;

²SEM = Standard error of the means;

³NS = Non-significant (p > 0.05), * = Significant (p < 0.05), ** = Significant (p < 0.01), *** = Significant (p < 0.001);

^{a-b}Means bearing different superscripts in a column differ (p < 0.05).

Treatment factors ¹	Performance of broiler birds										
	ILW (g/bird/d)	FLW (g/bird/d)	ADFI (g/bird/d)		ADG (g/bird/d)			FE			
N ₁ ×A ₁ ×V ₃	41.43	2156.67	17.25	113.33	74.90	20.95	70.66	50.78	0.82	1.58	1.28
N ₁ ×A ₀ ×V ₄	44.75	2167.62	17.75	114.00	75.50	19.45	75.93	53.34	0.91	1.53	1.28
N ₁ ×A ₁ ×V ₄	42.00	2266.43	17.25	113.33	74.90	20.86	73.75	52.59	0.82	1.52	1.24
SEM ¹²	0.28	14.65	0.19	0.25	0.23	0.16	0.69	0.41	0.01	0.01	0.01
Significance ¹³											
NaHCO ₃	NS	NS	***	***	***	NS	NS	NS	**	NS	NS
L-arg. + vitamin-C	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS
Vegetable oil	NS	*	**	**	**	**	NS	NS	***	NS	NS
NaHCO ₃ ×L-arg.×Veg.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
¹ N ₀ A ₁ V ₃ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;											
N ₁ A ₀ V ₃ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, vegetable oil 3%;											
N ₁ A ₁ V ₃ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;											
N ₀ A ₀ V ₄ = NaHCO ₃ 0%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;											
N ₀ A ₁ V ₄ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;											
N ₁ A ₀ V ₄ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;											
N ₁ A ₁ V ₄ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;											
² SEM = Standard error of the means;											
³ NS = Non-significant (p > 0.05), * = Significant (p < 0.05), ** = Significant (p < 0.01), *** = Significant (p < 0.001);											
^{a-b} Means bearing different superscripts in a column differ (p < 0.05).											

Carcass characteristics

Main as well as interaction effects of supplementing NaHCO₃, L-arginine + vitamin-C and vegetable oil appeared nil (p > 0.05) on the carcass characteristics of the experimental broiler birds (Table 5).

Table 5

Carcass characteristics of the broiler birds fed diets supplemented with various levels of NaHCO₃, L-arginine + vitamin-C and vegetable oils

Treatment factors ¹	Relative weight of the carcass components as % live weight											
	DP ²	BRW ³	BKW ⁴	TW ⁵	DW ⁶	SW ⁷	WW ⁸	NW ⁹	LW ¹⁰	GW ¹¹	PW ¹²	AFW ¹³
NaHCO ₃ (N)												
0.00%	62.05	16.72	11.00	7.58	6.99	3.57	4.22	2.14	1.85	2.58	0.45	1.31
0.10%	60.04	16.40	10.60	7.40	6.65	3.64	4.11	1.95	2.10	2.45	0.39	1.16
L-arginine + vitamin-C (A)												
0.00%	61.39	16.68	10.72	7.61	6.90	3.62	4.19	2.08	2.06	2.49	0.41	1.15
0.10%	60.70	16.44	10.88	7.38	6.73	3.58	4.14	2.01	1.89	2.53	0.43	1.32
Vegetable oil (V)												
3.00%	61.36	16.81	10.67	7.57	6.87	3.88	4.23	1.96	1.88	2.54	0.42	1.06
4.00%	60.73	16.32	10.93	7.41	6.77	3.33	4.10	2.12	2.08	2.49	0.42	1.41
N×A×V												
N ₀ ×A ₀ ×V ₃	63.22	17.57	10.76	7.71	7.21	3.92	4.34	2.08	1.98	2.51	0.46	0.98
N ₀ ×A ₁ ×V ₃	61.89	16.81	11.31	7.59	6.64	3.15	3.90	2.16	1.85	2.51	0.38	1.55
N ₀ ×A ₀ ×V ₄	61.59	16.38	11.18	7.65	6.87	3.75	4.04	2.01	1.73	2.72	0.51	1.23
N ₀ ×A ₁ ×V ₄	61.51	16.14	10.74	7.39	7.23	3.44	4.60	2.30	1.85	2.58	0.45	1.49
N ₁ ×A ₀ ×V ₃	60.15	16.25	10.40	7.43	6.87	4.07	4.46	2.07	1.96	2.58	0.35	0.86
N ₁ ×A ₁ ×V ₃	60.31	16.11	10.42	7.70	6.90	3.35	4.06	2.00	2.46	2.38	0.43	1.21
N ₁ ×A ₀ ×V ₄	60.49	17.03	10.34	7.50	6.52	3.75	4.08	1.70	1.84	2.34	0.35	1.17
N ₁ ×A ₁ ×V ₄	59.22	16.21	11.25	6.98	6.30	3.38	3.83	2.02	2.15	2.48	0.41	1.40
SEM ¹²	0.28	14.65	0.58	0.29	0.18	0.12	0.13	0.08	0.08	0.07	0.08	0.06
Significance ¹³												
NaHCO ₃	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
L-arg. + vitamin-C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Vegetable oil	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NaHCO ₃ ×L-arg.×Veg.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
¹ N ₀ A ₁ V ₃ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;												
N ₁ A ₀ V ₃ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, vegetable oil 3%;												
N ₁ A ₁ V ₃ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;												
N ₀ A ₀ V ₄ = NaHCO ₃ 0%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;												

Treatment factors ¹	Relative weight of the carcass components as % live weight											
	DP ²	BRW ³	BKW ⁴	TW ⁵	DW ⁶	SW ⁷	WW ⁸	NW ⁹	LW ¹⁰	GW ¹¹	PW ¹²	AFW ¹³
N ₀ A ₁ V ₄ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;												
N ₁ A ₀ V ₄ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;												
N ₁ A ₁ V ₄ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;												
² DP=Dressing percentage, ³ BRW=Breast weight, ⁴ BKW=Back weight, ⁵ TW=Thigh weight, ⁶ DW=Drumstick weight, ⁷ SW=Shank weight, ⁸ WW=Wing weight, ⁹ NW=Neck weight, ¹⁰ LW=Liver weight, ¹¹ GW=Gizzard weight, ¹² PW=Proventriculus weight, ¹³ AFW=Abdominal fat weight;												
¹⁴ SEM = Standard error of the means;												
¹⁵ NS = Non-significant (p > 0.05).												

Cardio-pulmonary morphometry

Supplementation of L-arginine + vitamin-C decreased (p < 0.01) the heart rate. Further, NaHCO₃ × L-arginine + vitamin-C × Vegetable oil additively interacted to decrease (p < 0.01) the weight of right and left ventricles in terms of heart weight, the ratio of RV:TV and heart rate but did not exceed the normal thresholds (Table 6).

Table 6

Cardio-pulmonary morphometry of the broiler birds fed diets supplemented with various levels of NaHCO₃, L-arginine + vitamin-C and vegetable oils

Treatment factors ¹	Cardio-pulmonary morphometry									
	HW ²	LW ³	RVW ⁴	LVW ⁵	RV/HW ⁶	LV/HW ⁷	RV/TV ⁸	RVT ⁹	RVD ¹⁰	HB ¹¹
NaHCO ₃ (N)										
0.00%	14.13	12.00	0.66	3.13	5.05	22.12	0.19	1.63	5.88	387.78
0.10%	13.13	11.75	0.66	2.88	4.69	21.90	0.17	1.50	5.00	384.78
L-arginine + vitamin-C (A)										
0.00%	13.50	13.63	0.68	2.88	5.00	21.82	0.19	1.63	5.75	390.00 ^a
0.10%	13.75	11.25	0.66	3.00	4.82	21.30	0.18	1.63	5.25	382.50 ^b
Vegetable oil (V)										
3.00%	13.38	12.25	0.63	2.88	4.67	21.50	0.17	1.63	5.38	383.28
4.00%	13.88	11.50	0.63	3.00	4.50	21.62	0.18	1.50	5.63	388.50
N×A×V										
N ₀ ×A ₀ ×V ₃	15.00	13.38	0.76	3.25	5.08	21.67	0.19	1.75	5.88	395.00
N ₀ ×A ₁ ×V ₃	13.75	12.50	0.58	3.13	4.18	22.73	0.16	1.50	5.88	385.00
N ₀ ×A ₀ ×V ₄	13.75	11.25	0.59	3.13	4.27	22.73	0.16	1.25	6.25	397.50
N ₀ ×A ₁ ×V ₄	13.75	10.88	0.79	2.88	5.73	20.91	0.22	2.13	5.63	372.50
N ₁ ×A ₀ ×V ₃	10.88	12.88	0.65	2.25	5.98	20.69	0.22	1.63	5.00	390.00
N ₁ ×A ₁ ×V ₃	13.75	10.38	0.70	3.00	5.09	21.82	0.16	1.50	4.75	362.50
N ₁ ×A ₀ ×V ₄	14.13	12.50	0.68	3.13	4.78	22.12	0.18	1.63	5.88	375.00
N ₁ ×A ₁ ×V ₄	13.75	11.25	0.60	3.13	4.36	22.73	0.19	1.25	4.63	410.00
SEM ¹²	0.48	0.43	0.03	0.11	0.23	0.29	0.01	0.10	0.23	5.89
Significance ¹³										
NaHCO ₃	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
L-arg. + vitamin-C	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
Vegetable oil	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NaHCO ₃ ×L-arg.×Veg.	NS	NS	NS	NS	**	**	**	NS	NS	**
¹ N ₀ A ₁ V ₃ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;										
N ₁ A ₀ V ₃ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, vegetable oil 3%;										
N ₁ A ₁ V ₃ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;										
N ₀ A ₀ V ₄ = NaHCO ₃ 0%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;										

Treatment factors ¹	Cardio-pulmonary morphometry									
	HW ²	LW ³	RVW ⁴	LVW ⁵	RV/HW ⁶	LV/HW ⁷	RV/TV ⁸	RVT ⁹	RVD ¹⁰	HB ¹¹
N ₀ A ₁ V ₄ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;										
N ₁ A ₀ V ₄ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;										
N ₁ A ₁ V ₄ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;										
² HW = Heart weight (g), ³ LW = Lung weight (g), ⁴ RVW = Right ventricle weight (g), ⁵ LVW = Left ventricle weight (g), ⁶ RV/HW = Right ventricle weight (% heart weight), ⁷ LV/HW = Left ventricle weight (% heart weight), ⁸ RV/TV = Right ventricle weight: total ventricle weight, ⁹ RVT = Right ventricle thickness (mm), ¹⁰ RVD = Right ventricle diameter (mm), ¹¹ HB = Heart beat/minute;										
¹² SEM = Standard error of the means;										
¹³ NS = Non-significant (p > 0.05), ** = Significant (p < 0.01);										
^{a-b} Means bearing different superscripts in a column differ (p < 0.05).										

Hemato-biochemical indices

Dietary NaHCO₃ increased (p < 0.05) TP and vegetable oil increased (p < 0.05) Hb and PCV (p < 0.05) as the main effect. The influence of L-arginine + vitamin-C appeared nil (p > 0.05) on all the hemato-biochemical indices. The NaHCO₃ × L-arginine + vitamin-C × Vegetable oil additively interacted to increase (p < 0.05) the triiodothyronine (T3) (Table 7).

Table 7

Blood pH, blood glucose (mg/dl), total cholesterol (TC, mg/dl), serum glutamic pyruvic transaminase (SGPT, U/L), serum glutamic oxaloacetic transaminase (SGOT, U/L), albumin (mg/dl), total protein (TP, mg/dl), triiodothyronine (T_3 , ng/dl), haemoglobin (Hb, g/dl) and packed cell volume (PCV, %) of the broiler birds fed diets supplemented with various levels of NaHCO_3 , L-arginine + vitamin-C and vegetable oils

Treatment factors ¹	Hemato-biochemical indices								
	Blood pH	Glucose (mg/dl)	TC (mg/dl)	SGPT (U/L)	SGOT (U/L)	TP (g/L)	T_3 (ng/dl)	Hb (g/dl)	PCV (%)
NaHCO ₃ (N)									
0.00%	6.7	259.3	141.8	14.6	23.7	32.8 ^a	96.0	14.5	31.4
0.10%	6.8	289.5	146.3	14.2	23.3	40.4 ^b	108.0	14.5	31.2
L-arginine + vitamin-C (A)									
0.00%	6.7	257.5	145.2	13.8	24.0	37.5	105.0	14.8	31.8
0.10%	6.8	291.3	152.8	15.0	23.0	35.7	98.0	14.3	30.9
Vegetable oil (V)									
3.00%	6.8	272.4	147.0	14.7	23.6	37.8	94.0	13.0 ^a	29.1 ^a
4.00%	6.8	276.4	151.1	14.2	23.4	35.4	106.0	16.0 ^b	33.6 ^b
NxAxV									
N ₀ ×A ₀ ×V ₃	6.7	244.9	142.1	13.2	23.0	38.7	92.0	13.3	30.3
N ₀ ×A ₁ ×V ₃	6.8	263.1	148.0	12.9	22.1	29.8	95.0	12.2	27.3
N ₀ ×A ₀ ×V ₄	6.8	237.5	140.0	15.1	26.3	28.4	91.0	17.7	35.2
N ₀ ×A ₁ ×V ₄	6.6	291.6	137.0	17.4	23.2	34.4	104.0	15.0	32.9
N ₁ ×A ₀ ×V ₃	6.7	261.3	147.7	16.1	24.9	47.3	129.0	12.6	28.4
N ₁ ×A ₁ ×V ₃	6.9	320.2	150.2	16.5	24.5	35.2	74.0	13.9	30.4
¹ N ₀ A ₁ V ₃ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;									
N ₁ A ₀ V ₃ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, vegetable oil 3%;									
N ₁ A ₁ V ₃ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;									
N ₀ A ₀ V ₄ = NaHCO ₃ 0%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;									
N ₀ A ₁ V ₄ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;									
N ₁ A ₀ V ₄ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;									
N ₁ A ₁ V ₄ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;									
² SEM = Standard error of the means;									
³ NS = Non-significant (p > 0.05), * = Significant (p < 0.05);									
a ^{-b} Means bearing different superscripts in a column differ (p < 0.05).									

Treatment factors ¹	Hemato-biochemical indices								
	Blood pH	Glucose (mg/dl)	TC (mg/dl)	SGPT (U/L)	SGOT (U/L)	TP (g/L)	T ₃ (ng/dl)	Hb (g/dl)	PCV (%)
N ₁ ×A ₀ ×V ₄	6.7	286.2	151.2	10.9	21.9	35.5	107.0	15.3	33.2
N ₁ ×A ₁ ×V ₄	7.0	290.3	176.1	13.4	22.0	43.4	121.0	16.1	32.9
SEM ²	0.07	9.54	4.05	0.72	5.82	1.82	4.6	0.69	1.01
Significance ³									
NaHCO ₃	NS	NS	NS	NS	NS	*	NS	NS	NS
Additives	NS	NS	NS	NS	NS	NS	NS	NS	NS
Vegetable oil	NS	NS	NS	NS	NS	NS	NS	*	*
NaHCO ₃ ×L-arg.×Veg.	NS	NS	NS	NS	NS	NS	*	NS	NS
¹ N ₀ A ₁ V ₃ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;									
N ₁ A ₀ V ₃ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, vegetable oil 3%;									
N ₁ A ₁ V ₃ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 3%;									
N ₀ A ₀ V ₄ = NaHCO ₃ 0%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;									
N ₀ A ₁ V ₄ = NaHCO ₃ 0%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;									
N ₁ A ₀ V ₄ = NaHCO ₃ 0.1%, L-arginine 0% + Vitamin-C 0%, Vegetable oil 4%;									
N ₁ A ₁ V ₄ = NaHCO ₃ 0.1%, L-arginine 0.05% + Vitamin-C 0.05%, Vegetable oil 4%;									
² SEM = Standard error of the means;									
³ NS = Non-significant (p > 0.05), * = Significant (p < 0.05);									
^{a-b} Means bearing different superscripts in a column differ (p < 0.05).									

Discussion

According to Wideman et al. (2013), the left ventricle of the heart drives the oxygenated blood to the whole body to support maintenance, growth and productivity of the broiler birds. The volume of blood pumped by the left ventricle each minute is known as the cardiac output which in clinically healthy bird is propelled entirely by the right ventricle through the lungs at relatively low pulmonary arterial pressure to reduce the risk of fluid filtration into the gas exchange spaces (Martinez-Lemus et al., 1999, 2003; Odom et al., 2004; Wideman et al., 2013). This low pressure sustains as long as pulmonary vasculature upholds a suitably low resistance to the blood flow. However, genetically modified broiler birds with improved growth potentials, feed efficiency and relatively higher muscle to organ ratio require increased blood flow to satisfy their ever increasing metabolic demand. In order to supply increased blood flow, the right ventricle consistently initiate elevated heart beats leading to tachycardia.

Under critical episodes of progressive tachycardia, the pulmonary arterial pressure accelerates the rate of blood flow which stimulates RBC racing too fast through the pulmonary vasculature (Wideman et al., 2013). Due to increased circulation rate, RBC gets shorter than the normal transit time at the gas exchange surfaces for complete diffusive exchange of O₂ and CO₂ and fails to achieve full blood-gas equilibrium (Henry and Fedde, 1970; Powell et al., 2000; Wideman et al., 2013). The unsaturated blood

then enters the systemic flow with a lower than usual partial pressure of O₂ and a higher partial pressure of CO₂ resulting hypoxemia (Peacock et al. 1990; Reeves et al. 1991; Julian and Mirsalimi 1992; Wideman and Kirby 1996; Roush et al. 1997).

Hypoxemia further triggers polycythemia by stimulating erythropoietin which increases hematocrit to enhance the O₂ carrying capacity of blood (Shlosberg et al., 1996; Scheele et al., 2003; Tekeli, 2014). Progressive elevation of hematocrit and the reduced deformability of the rapidly produced immature erythrocytes consistently increase blood viscosity (Gregersen et al., 1969; Hakim and Macek, 1988), thereby, thrombotic occlusion (Currie, 1999; Baghbanzadeh and Decuypere, 2008; Wideman et al., 2013) and finally ascitic heart failure (Fedde and Wideman 1996; Shlosberg et al. 1996, 1998; Wideman et al. 1998). Consistent tachycardia as well as polycythemia thus indirectly bears indications of ascites which impairs performance, carcass and health of the birds as measured by hemato-biochemical indices.

Performance

Our study indicated that, the addition of L-arginine + vitamin-C in the diet of the broiler chicken significantly improved feed intake from 3–5 weeks. Similar result was reported by Al-Daraji and Salih (2012). Supplementation of L-arginine + vitamin-C in the mash feed resulted better weight gain during last two weeks of rearing which is in line with the findings of other studies (Njoku 1986; Al-Daraji and Salih 2012). Significantly higher weight gain might have been due to increased feed intake. It is well established that, vitamin-C reduces the extents of oxidative heat stress efficiently (Masad 2012; Dalia et al. 2018) which is the underlying cause of increased feed intake and weight gain (Njoku, 1986) in the broiler birds in our study. Further, these two additives improve the immune status of the birds which could have helped in better performance of the commercial broiler birds used in current study (Masad 2012; Dalia et al. 2018). Similarly, L-arginine, an indispensable amino acid for poultry as well as human which improves the release of growth hormone and muscle growth that could have contributed to the better feed intake and daily weight gain in our study (Stevens et al. 2000).

In the present study, incorporation of vegetable oil in the diet of boiler birds significantly improved average daily gain and FCR during the initial period. These findings coincide the results of Attia et al. (2020) although differ from the observation of Ayed et al. (2015). One of the objectives of the current study was to see the interaction effect of NaHCO₃, L-arginine + vitamin-C and vegetable oil in accordance with the performance of broiler chicken. Interaction effect of NaHCO₃ and L-arginine + vitamin-C showed significant rise in feed intake in the last two weeks of the trial. As stated above, effect of reducing heat stress due to addition of additives present in the diet could be one of the reasons behind the improved feed intake (Masad 2012; Dalia et al. 2018). Accordingly, at the same time, NaHCO₃ on diet apparently had association with the increasing feed intake and the reason appeared to be due to the bicarbonate ion which was associated with an increased water intake (Balnave and Gorman, 1993). Similar interaction effects were seen in the birds fed diet containing combination of L-arginine + vitamin-C and vegetable oil. As mentioned earlier, vitamin-C in the test diets helped to reduce the environmental stress (Masad 2012; Dalia et al. 2018). Further, the vegetable oils in the diet met the increasing demand of energy (Attia et al., 2020), especially during the finisher stage. These effects might be the reason of rise in energy intake in the broiler birds fed diets containing both the L-arginine + vitamin-C and the vegetable oil.

From the result of our study, it is obvious that, average feed intake of the birds fed NaHCO₃ supplemented diet throughout the trial increased substantially which agrees with the previous findings (Arp et al., 1984; Roussan et al., 2008; Osman et al., 2015). The increased feed intake, however, might be a result of reduced heat stress in broilers caused by NaHCO₃ (Roussan et al., 2008). In our study, NaHCO₃ also improved the average daily gain of birds in the last two weeks which is aligned with the previous studies (Roussan et al., 2008; Osman et al., 2015; Saker et al., 2020). Better feed intake and daily gain of broilers fed NaHCO₃ ultimately resulted significantly better FCR in the last two weeks which is supported by the previous studies (Hooge et al., 2000; Nidgundi et al., 2007).

Carcass characteristics

All the carcass criteria, i.e., dressing percentage, weight of breast, thigh, wings, drumstick and other organs were unaffected among dietary treatments in the current study. These findings agree with the results of some previous studies (Petrolli et al., 2016) although differ with the results reported by others elsewhere (Hooge et al., 1999; Ogunwole et al., 2014). Here, in our study,

liver weight was found lower in broiler birds fed experimental diets containing L-arginine + vitamin-C that further coincides with the findings of Susantoputro et al. (2014). The interaction effects of test ingredients on carcass merit were also found non-significant between diets.

Cardio-pulmonary morphometry

Dietary supplementation of 130% arginine of the requirement improved the intestinal morphology and performance and decreased the cold induced ascitic mortality in broiler chickens (Abdulkarimi et al., 2019). In a previous study, additional arginine administered *in ovo* or in the feed reduced the vulnerability of broilers to pulmonary hypertension (Saki et al., 2013). Additionally, broiler chickens reared at high altitude and predisposed to pulmonary hypertension and ascites were partly explained by arginine supplementation (Khajali et al., 2011). Supplemental arginine improved the pulmonary vascular performance of hypoxic broiler birds and its outcomes were further enhanced by the addition of the vitamin-C. Arginine and antioxidant vitamins might have taken part in synergistic functions to improve nitric oxide bioavailability as potent natural vasodilator and lessen oxidative damage, thus increasing cardiopulmonary performance (Bautista-Ortega and Ruiz-Feria, 2010).

It was further reported that, arginine or guanidinoacetic acid supplementation of diets did not affect gross response of birds under standard temperature, but addition of arginine to the diet significantly reduced the incidence of cold stress on performance, gut development and ascites syndrome (Kodambashi et al. 2017). In fact, L-arginine is a substrate for nitric oxide, which acts as a potent endogenous pulmonary vasodilator that substantially reduces the right ventricle: total ventricle ratio (Wideman et al. 1995). Consistent with these points, addition of L-arginine and thereby, reduced susceptibility of the birds against tachycardia and polycythemia evident in our study is likely.

Rostami et al. (2016) reported that, the birds receiving flax oil had significantly higher serum concentration of nitric oxide. The right-to-total ventricle weight ratio (RV/TV) and death from pulmonary hypertension were significantly ($p < 0.05$) declined in birds that fed on flax oil. It was argued that, n-3 fatty acids could significantly lessen RV:TV and PHS death in birds by escalating circulatory level of nitric oxide and suppressing hepatic lipogenesis. In another study, administration of flax oil reduced blood viscosity, right ventricular hypertrophy, hematocrit and hemoglobin content and improved erythrocyte deformability by increasing the quantity of unsaturated fatty acids in the erythrocyte membranes and thereby decreased ascites induced mortality (Walton et al., 1999, 2001). With reference to these points, addition of vegetable oil in the present study might have contributed additional n-3 fatty acids responsible for improving RV:TV and thereby no evidence of tachycardia in the experimental birds fed test diets in our study.

Supplementation of vitamin C substantially improved cellular integrity and reduced incidence of mortality by ascites in a previous study (Roch et al., 2000). Similarly, addition of vitamin C reduced the possibility of thick-walled peripheral vessels in the lungs and thereby, the incidence of ascites (Xiang et al., 2002). Vitamin-C reduces muscularization of the pulmonary arterioles by scavenging oxygen-derived free radicals thus lower the number of thick-walled peripheral vessels to decrease the resistance to blood flow in the pulmonary vessels of broilers (Hassanzadeh Ladmakhi et al., 1997). The levels of α -tocopherol and γ -tocopherol were decreased in the mitochondria of an ascitic broiler, suggesting inadequate reactive oxygen scavenger in the primary site of energy transduction (Cawthorn et al., 2001). It could therefore be inferred that, addition of L-arginine + vitamin-C in the form of feed additive efficiently stabilized RV:TV, prevented tachycardia and polycythemia in the experimental birds used in our study.

Hemato-biochemical indices

A decline in blood pH lowers the oxygen affinity of haemoglobin, while increased blood pH increases oxygen affinity to increase haemoglobin saturation in the lungs (Isaacks et al., 1986). Thus, increased blood pH can improve the loading capacity of oxygen by haemoglobin in the lungs due to the Bohr effect. Accordingly, feeding bicarbonate supplemented diets result in a decrease in pulmonary hypertension (Barer et al. 1966; Balnave and Gorman 1993; Squires and Julian 2001). Perhaps, this is the reason behind the absence of tachycardia and thereby polycythemia in the experimental birds used in the present study.

The majority of commercial meat birds are fed crumbled or pelleted diets to attain utmost growth and feed efficiency. Feeding mash diet reduces growth rate. Broiler birds that consumed pellet feed had frequently been shown to have higher incidence of tachycardia than the birds that consumed the same diet in mash form (Shlosberg et al., 1992; Bölükbasi et al., 2005). Unlike

pellets, mash diets are not subjected to steam conditioning prior to pelleting. Thus, mash diets are less palatable and have reduced microbial and enzymatic digestibility. Consequently, mash diets have far less susceptibility for tachycardia and polycythemia in broiler birds as evident in our study.

In ascitic birds, Dahiya et al. (2001) reported a significant rise in haemoglobin, packed cell volume, total erythrocyte count, serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphates and phosphorus and marked fall in serum total proteins, albumin globulin ratio (A:G), calcium and sodium although erythrocyte indices, i.e., mean corpuscular volume, and mean corpuscular haemoglobin concentration remained unchanged. Consistent results were reported elsewhere indicating significant increase in haemoglobin concentration (Maxwell et al., 1986; Dahiya et al., 2001; Ipek and Sahan, 2006; Wang et al., 2012; Tekeli, 2014), higher values for haematocrit and total erythrocyte count (Maxwell et al. 1986; Dahiya et al. 2001; Ipek and Sahan 2006; Reza et al. 2008; Hafshejani et al. 2012; Wang et al. 2012; Tekeli 2014) in ascitic birds.

Similarly, marked decrease in plasma proteins, i.e., total proteins, albumin and albumin:globulin ratio (Biswas et al., 1995; Dahiya et al., 2001; Daneshyar et al., 2009; Wang et al., 2012) and increase in serum bilirubin, AST, ALT, LDH, ALP (Dahiya et al., 2001; Reza et al., 2008), plasma lipids and total cholesterol (Biswas et al., 1995; Wang et al., 2012) were reported further in ascitic birds. Additionally, the HDL cholesterol significantly decreased reflecting progressive cardiomyopathy and subsequently cardiac failure in previous studies. Since direct evidences are scant, relying upon above reports, it can indirectly be inferred that the birds susceptible for tachycardia and polycythemia will reveal similar symptoms. Interestingly, all above hemato-biochemical parameters as well as heart beat persisted within standard range (Bounous and Stedman, 2000) exhibiting no unusual changes thereby least possibility of tachycardia or polycythemia either in control or test groups in our study.

Conclusion

Plant based high energy mash diets are not susceptible to tachycardia and polycythemia. Addition of NaHCO₃, L-arginine + Vitamin-C ameliorate propensity of tachycardia and polycythemia without deteriorating performance, carcass characteristics and hemato-biochemical indices of the commercial broiler birds in a dose dependent manner.

Declarations

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Conflicts of interest/Competing interests

None.

Availability of data and materials

All the data used in the manuscript exclusively belongs to the mentioned authors.

Code availability

None.

Author's contribution

Dr. Md. Emran Hossain conceived the study, analyzed data, interpreted the results and finalized the draft. Dr. Nasima Akter procured research grant, conducted the animal trial, ran the immunization program, collected data and prepared the initial draft. All authors read and approved the final manuscript.

Ethics approval

The entire experimental protocol was approved by the animal welfare law in Bangladesh (Memo No. CVASU/Dir(R&E)EC/2021/244(3)).

Consent for publication

Yes.

Consent to participate

Yes.

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