The effect of different levels of sodium chloride on round heart disease in the turkey

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Summery

Round heart disease is a disease of unknown etiology in turkey. The objective of this study was to examine the effect of different amounts of sodium chloride in inducing cardiomyopathy in turkey poults. There are some reports that hypoxia and furazolidone can induce cardiomyopathy in turkey. Two hundred 2-day-old turkey poults were wing-banded and placed randomly into 6 groups and 24 replicates (4 replicates for each group). The 6 groups received commercial turkey starter diet contain 0.12, 0.17, 0.22, 0.27, 0.32 and 0.37% of sodium, respectively. They had access to food and water ad libitum. Suffering and dead birds were weighed and examined for post-mortem study. Live body weight of turkey poults measured individually on days 1, 2, 5 and 19. To study the hearts for presence of cardiomyopathy, on the day 19, all the poults were weighed and killed by cervical dislocation. The pieces of the hearts were placed into numbered tubes, flash frozen by liquid nitrogen and stored at -70ºC for myoglobin and hemoglobin microassay. The assay was based on spectrophotometry and separated myoglobin and hemoglobin by ammonium sulphate extraction. For determination of cardiomyopathy the ratio of the inner cavity of left ventricle to the total diameter of the left ventricle (IC_{lv}/TD_{lv}) was calculated. Myopathic ratio for groups 1, 2, 3, 4, 5 and 6 were 0.1, 0.29, 0.3, 0.34, 0.35 and 0.36, respectively. Turkey poults which received excessive salts drank more water which produced volume overload, more cardial muscle activity, needed more oxygen and were more likely to develop myopathy. Cardiac muscle myoglobin and hemoglobin microassay showed that there was no significant relationship between the amount of myoglobin and hemoglobin with the amount of diet sodium.

Key words: Round heart disease, Sodium chloride, Myoglobin, Turkey poults

Introduction

Spontaneous turkey cardiomyopathy (STC) has commonly been called round heart disease (RHD) and less commonly is known as the cardiohepatic syndrome. It is desirable that the use of the term “round heart disease” be discontinued, as the syndrome in turkeys is different in many respects from RHD in chickens (Magwood and Bray, 1962). The etiology of cardiomyopathy is still unknown (Julian, 1993). Spontaneous cardiomyopathy can be characterized by stunted growth, difficulties in breathing at normal exercise levels and a considerably enlarged, round heart. Spontaneous cardiomyopathy occurs most frequently in inbred, small broad breasted white strains of turkeys (Czarnecki, 1984). In fact, cardiomyopathy develops in 70% of inbred flocks at very early stages of life (Marin *et al*., 1996). Investigators have conducted numerous studies to find out what kind of stress is involved in the development of cardiomyopathy (Julian, 1998). RHD occurs spontaneously (Magwood and Bray, 1962) or it can be induced by the administration of furazolidone to turkey poults (Jankus *et al*., 1972). Clinical observation indicates that the incidence of spontaneous cardiomyopathy is increased at high altitude, cold weather and in rapidly growing poults. It is probable that an increased oxygen requirement may increase the incidence of spontaneous cardiomyopathy (Julian, 1993). This study was conducted to examine the effect of different amounts of sodium chloride in inducing cardiomyopathy in turkey poults.

Materials and Methods

Two hundred 2-day-old turkey poults were weighed, wing-banded and randomized into 6 groups and 24 replicates (4 replicates

for each group). Turkey poults were grown up in a battery in the Animal and Poultry Science building at the University of Guelph. They were raised for 19 days on commercial turkey starter ration (Table 1). Turkeys had access to food and water ad libitum. Each group received commercial turkey starter with a level of sodium as is showed in Table 2. Suffering and died birds were weighed and examined for post-mortem study. Presence of cardiomyopathy and a summery of necropsy were reported.

Turkey poults were weighed on days 1, 2, 5 and 19. To study the hearts for presence of cardiomyopathy, on the day 19, all the poults were weighed and killed by cervical dislocation. The heart of killed animals was immediately removed and the atria and major vessels were cut and weighed to determine the heart weight. To identify the presence of cardiomyopathy, the heart of each turkey poult was cross sectioned and the inner cavity and the total diameter of left ventricle were measured by a coulisse (Lax *et al*., 1988).

The pieces of the hearts were placed into numbered tubes, flash frozen by liquid nitrogen and stored at -70°C for myoglobin and hemoglobin microassay. The heart pieces of 8 birds from each group were sampled at random to assay myoglobin and hemoglobin according to the following protocol:

Two grs of cardiac muscle, that was stored at -70°C was weighed and added to a centrifuge tube of 8 ml of ice cold buffer containing 80 mM KCl with 50 mM tris-HCl (pH = 7.8) and kept on ice. The samples were homogenized and centrifuged at 8500 rpm for 20 min at 4°C. About 1.5 ml of the clear supernatant was removed and filtered through a 0.45 µm pore milipore filter to 16 ×100 mm culture tube and covered with a plastic cap for hemoglobin analysis. Remaining supernatant was transferred to another centrifuge tube and it's volume recorded. Ammonium sulphate in 0.525 g/ml were added to the supernatant to precipitate hemoglobin and well the vortex up and then centrifuged at the above-mentioned speed for 20 min. Tubes were removed from centrifuge carefully without shaking and the clear supernatant filtered through a 0.45 µm pore milipore filter in 16×100 mm culture tubes, then covered with a cap for myoglobin measurement.

For myoglobin assay, 0.2 ml of the filtrate in which hemoglobin has been precipitated was removed and 0.76 ml of deionized water, 0.04 ml of 50 mM orthotolidone in 0.1 N HCl, which is made fresh daily and 0.2 ml of 1 M tertiary butyl hydroxides were added to it in duplicate. Vortex was welled up and stood for 10 min at room temperature. A series of dilution were made ranging from 1 µg to 20 µg, varying in standard and tris-KCl solution. Equine myoglobin at a concentration of 100 µg/ml was used as standard. The absorbances were measured at 630 nm by a spectrophotometer.

For hemoglobin assay, 0.4 ml of prior clear supernatant was removed and filtered through a 0.45 µm pore milipore filter and then 1.6 ml of 80 mM KCl with 50 mM tris-HCl buffer were added to it in duplicate. 20 µl of 10 mM potassium ferricyanide were added, vortex was welled and stood at room temperature for 10 min. A series of dilution ranging from 50 µg to 250 µg of hemoglobin and tris-KCl were used as standard. Two-hundred and fifty µg/ml of bovine hemoglobin was used as standard. The absorbances were measured at 420 nm by a spectrophotometer (O'Brien *et al*., 1992).

All the measured data for different groups were subjected to one-way analysis of variance using the general linear models procedure of SAS. For statistical evaluation of differences between the groups, the method of LSD was used (SAS Institute, 1985).

Results

Over the 19 days of the study, 0, 3, 0, 2, 0 and 0 poults died in groups 1, 2, 3, 4, 5 and 6, respectively. To eliminate suffering birds from the experiment, 5, 1, 2, 0, 2 and 3 turkey poults from groups 1, 2, 3, 4, 5 and 6 were removed, respectively; weighed and necropsied.

On the 19th day, 184 turkey poults were killed by cervical dislocation for examination for detecting cardiomyopathy. The effects of salt levels on weight gain, heart weight, total diameter of the left ventricle (TD_{lv}), inner cavity of the left ventricle (IC_{lv}), myopathic ratio (IC_{lv}/TD_{lv}), hemoglobin and myoglobin

are showed in Table 3. Salt induced RHD or STC. As depicted in Fig. 1, generally the heart mass was correlated with body mass. There were, however, a few exceptions; there were turkeys that had disproportionately much larger hearts as compared to their body size. These turkeys were most likely suffered from cardiomyopathy and were used for further analysis for diagnosis of cardiomyopathy. For determination of cardiomyopathy the ratio of IC_{lv}/TD_{lv} was calculated (Fig. 2). Myopathic ratio increased by increasing of sodium concentration in the diet.

Discussion

This study provides comprehensive characterization of cardiac muscle changes occurring in different levels of sodium chloride-induced cardiomyopathy. The curve of myopathic ratio between 0.12% and 0.17% of sodium concentration showed a linear model and was much clear, but myopathic ratio between 0.17% and 0.37% of sodium concentration showed plateau model increasing (Fig. 3).

Based on our findings, spontaneous turkey cardiomyopathy was induced by

Table 1: Commercial turkey poults starter formula used for the experiment, during 19 days of growing

Code	Ingredient	Weight (Kg)	%
1	Corn	423.80	42.380
7	Soybean meal	440.13	44.013
11	Meat meal	60.00	6.000
14	Animal vegetable blend of fat	30.50	3.050
15	DL-methionine	3.45	0.345
16	L-lysine	1.15	0.115
17	Salt	1.12	0.112
18	Limestone	12.75	1.275
19	Dicalcium phosphate	17.10	1.710
20	Vit-Min Pmx	10.00	1.000
	Formula totals	1,000.00	

Table 2: The six diets with different levels of sodium used for the experiment during 19 days of raising

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Basal	30.000	29.962	29.924	29.886	29.845	29.810
Salt		0.038	0.076	0.114	0.152	0.190
Total (kg)	30.000	30.000	30.000	30.000	30.000	30.000
Sodium (%)	0.12	0.17	0.22	0.27	0.32	0.37

Table 3: Body weights for days 1, 2, 5 and 19, hearts weight, total diameter of the left ventricle (TD_{lv}), inner cavity of the left ventricle (IC_{lv}), the ratio of IC_{lv}/TD_{lv}, hemoglobin and myoglobin of birds. Values are expressed as Mean ± SD. Within a column values with different superscripts are differ significantly ($P<0.05$)

Group	Day-1 Wt/gr	Day-2 Wt/gr	Day-5 Wt/gr	Day-19 Wt/gr	Heart Wt/gr	TD_{lv}	IC_{lv}	IC_{lv}/TD_{lv}	Hemo-globin g/100g	Myog-lobin mg/100g
1	59.8± 4.27^{ab}	70.7± 4.5^{ab}	137.6± 15.4^{b}	556.1± 59.03^{ab}	3.21± 0.42^{a}	13± 2.1^{cd}	1.31± 1.6^{d}	0.1± 0.14^{c}	1.4± 0.14^{c}	6.59± 6.44^{c}
2	60.1± 4.19^{ab}	72.8± 4.17^{a}	146.4± 12.7^{a}	576.1± 58.04^{a}	3.36± 0.34^{a}	14± 0.9^{bc}	4.06± 1.78^{c}	0.29± 0.13^{b}	3.73± 0.49^{a}	14.62± 7.12^{c}
3	60.4± 5.0^{a}	70.6± 5.94^{ab}	141± 17.0^{ab}	561.1± 69.08^{ab}	3.33± 0.41^{a}	15± 2.6^{b}	4.5± 1.93^{abc}	0.3± 0.12^{ab}	1.86± 0.65^{b}	108.3± 29.21^{ab}
4	59.1± 4.40^{ab}	69.7± 5.05^{b}	141± 14.0^{ab}	563.1± 53.05^{a}	3.37± 0.44^{a}	16.3± 3.5^{a}	5.33± 1.98^{a}	0.34± 0.15^{ab}	1.66± 0.38^{b}	70.99± 57.18^{b}
5	57.8± 3.04^{b}	70.6± 5.08^{ab}	143± 14.4^{ab}	553.3± 49.78^{ab}	3.26± 0.49^{a}	14± 2.9^{b}	5.09± 1.78^{ab}	0.35± 0.12^{ab}	1.06± 0.16^{c}	11.62± 8.91^{c}
6	59.7± 4.70^{ab}	70.4± 6.54^{ab}	142± 18.9^{ab}	532.4± 74.25^{b}	3.35± 0.49^{a}	14± 1.5^{bc}	5.15± 1.63^{ab}	0.36± 0.1^{a}	1.05± 0.172^{c}	132.6± 67.32^{a}

Fig. 1: A graph of heart mass vs total body mass of turkey poults on the 19th day of age

Fig. 2: A graph of the diameter of the inner cavity of left ventricle to the total diameter of the left ventricle, expressed as a ratio vs the frequency, found in the population of turkeys

Fig. 3: The curve of myopathic ratio vs different levels of sodium in the turkey poults diet

volume overload secondary to excessive salt intake. Myopathic ratio for groups 1, 2, 3, 4, 5 and 6 were measured 0.1, 0.29, 0.3, 0.34, 0.35 and 0.36, respectively. Turkey poults with excessive salts drink more water and this produce volume overload, more cardiac muscle activity, more oxygen requirement and cause more myopathy (Fig. 3). Cardiomyopathy is a disease involving enlargement and degeneration of the heart, leading to spontaneous death in young turkeys. It is possible that some young poults do not have enough oxygen available in their myocytes to maintain cardiac function and to allow the multiplication of ventricular myocytes that continues after hatching (Julian, 1990; Mirsalimi *et al*., 1990; Julian, 1991). Hypoxic conditions and the increased oxygen requirement for rapid growth would reduce the oxygen supply to myocytes. Low temperatures may increase the incidence of STC (Czarnecki, 1984). Cold increases the metabolic rate and oxygen requirement and excessive heat, particularly in young poults that have poor thermoregulatory system would also increase the metabolic rate and might increase the incidence of STC (Dunnington and Siegel, 1984; Julian *et al*., 1989). So far, few models have been proposed suggesting the role of decreasing levels of manganese superoxide dismutase (MnSOD) in the pathogenesis of cardiomyopathy in different animals and turkeys. The main source of superoxide in the heart is a by-product of the enzymes of the respiratory chain in the mitochondria. Heart tissue is rich in mitochondria. Mitochondria produce superoxide anions that are scavenged by MnSOD. Dysfunction of MnSOD scavenger system could cause therefore damage heart tissue (Marin *et al*., 1996). Insufficient ventricular myocyte myoglobin might also be involved, as it transfers oxygen to the mitochondria for ATP synthesis. Turkeys have lower myocardial myoglobin than chickens. Chickens and turkeys both have much lower myocardial myoglobin than dogs (O'Brien *et al*., 1992).

According to the results of the present study, there was no significant relationship between the amount of myoglobin and hemoglobin with the amount of sodium in diet. For myoglobin and hemoglobin assay, the heart pieces of 8 birds from each group were sampled, because the microassay for 184 samples was expensive and time-consuming. Therefore, the variable amount of myoglobin may be attributed to the methods of sampling and microassay that were performed for myoglobin analysis.

Myoglobin functions as an intracellular oxygen reservoir to protect the mechanical function of striated muscle from hypoxia (Braunlin *et al*., 1986; Bailey and Driedzic, 1986). It acts as an oxygen shuttle, facilitating the diffusion of oxygen from the capillary to the mitochondria by intracellular translation movement down its concentration gradient (Livingston *et al*., 1983; Gayeski and Honig, 1986). Myoglobin content is known to be increased by high-altitude-induced hypoxia in both man and animals, by physical training of animals, although it is not apparent in man and muscle fiber transformation (Millikan, 1939; Sylven *et al*., 1984). Studies on the role of alterations in myoglobin activity in the pathogenesis of skeletal and cardiac muscle disease are rare. However, it has been showed that myoglobin inhibition may result in decreased oxygen uptake, oxidative phosphorylation and mitochondrial ATP production, tension development and relaxation. Recently, myoglobin has implicated as a free radical generator resulting in lipoperoxidation during ischemia-reperfusion injury of myocardium (Arduino *et al*., 1990). Assay for quantification of myoglobin in striated muscle must distinguish this protein from hemoglobin of blood since the two tissues cannot be completely separated. Myoglobin is an intracellular protein, whereas hemoglobin is extracellular. These proteins may be physically or chemically distinguished on the basis of differences in their spectral absorption after derivatization, molecular weight, size, isoelectric points or antigenicity (Penn, 1979).

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References

1- Arduino, A; Eddy, L and Hochstein, P (1990). Detection of ferryl myoglobin in the isolation ischemic rat heart. Free Radical. Biol. Med., 9: 511-513.

2- Bailey, JR and Driedzic, WR (1986). Function of myoglobin in oxygen consumption by isolated perfused fish hearts. Am. J. Physiol., 251: R1144-R1150.

3- Braunlin, EA; Wahler, GM; Swayze, CR; Lucas, RV and Fox, IJ (1986). Myoglobin facilitated oxygen diffusion maintains mechanical function of mammalian muscle. Cardiovasc. Res., 20: 627-636.

4- Czarnecki, CM (1984). Review: cardiomyopathy in turkeys. Comp. Biochem. Physiol., 77A: 591-598.

5- Dunnington, EA and Siegel, PB (1984). Thermoregulation in newly hatched chicks. Poult. Sci., 63: 1303-1313.

6- Gayeski, TEJ and Honig, CR (1986). O2 gradient from sarcolemma to cell interior in red muscle at maximal VO2. Am. J. Physiol., 251: H789-H799.

7- Jankus, EF; Noren, GR and Staley, NA (1972). Furazolidone-induced cardiac dilatation in turkeys. Avian Dis., 16: 958-961.

8- Julian, RJ (1990). Cardiovascular disease. In: Jordan, FTW (Ed.), *Poultry diseases*. (3rd. Edn.), London, Balliere Tindall, PP: 345-353.

9- Julian, RJ (1991). The effect of management and environment on cardiovascular disease in poultry. *Proc. 42nd North Central Avian Disease Conferences*. Des Moines, Iowa. PP: 38-42.

10- Julian, RJ (1993). Ascites in poultry. Avian Pathol., 22: 419-454.

11- Julian, RJ (1998). Rapid growth problems: ascites and skeletal deformities in broilers. Poult. Sci., 77: 1773-1780.

12- Julian, RJ; McMillan, I and Quinton, M (1989). The effect of cold and dietary energy on right ventricular hypertrophy, right ventricular failure and ascites in meat-type chickens. Avian pathol., 18: 675-684.

13- Lax, D; Zhang, S; Li, Y; William, L; Berry, JM; Elsperger, J; Staley, NA; Noren, GR and Einzig, S (1988). Reduced lipid peroxidation in dilated hearts of cardiomyopathic turkeys. Cardiovasc. Res., 22: 826-832.

14- Livingstone, DJ; La Mar, GN and Brown, WD (1983). Myoglobin diffusion in bovine heart muscle. Sciences. 220: 71-73.

15- Magwood, SE and Bray, DF (1962). Disease condition of turkey poults characterized by enlarged and rounded hearts. Can. J. Com. Med., 26: 268-272.

16- Marin, GJ; Ananthakrishnan, R; Pierpont, ME and Goldenthal, MJ (1996). Mitochondrial dysfunction in spontaneous inbred turkey cardiomyopathy. Biochem. Mol. Biol. Intern., 38: 1087-1093.

17- Millikan, GA (1939). Muscle hemoglobin. Physiol. Rev., 19: 503-523.

18- Mirsalimi, M; Qureshi, FS; Julian, RJ and O'Brien, PJ (1990). Myocardial biochemical changes in furazolidone-induced cardiomyopathy of turkeys. J. Comp. Pathol.,

102: 139-147.

19- O'Brien, PJ; Shen, H; McCucheon, LJ; O'Grady, M; Byrne, PJ; Ferguson, HW; Mirsalimi, M; Julian, RJ; Sargeant, JM; Tremblay, RRM and Blackwell, TE (1992). Rapid, simple and sensitive microassay for skeletal and cardiac muscle myoglobin and hemoglobin: use in various animals indicates functional role of myohemoproteins. Mol. Cell. Biochem., 112: 45-52.

20- Penn, AS (1979). Myoglobin and myoglobinuria. In: Vinken, PJ and Bruyn, GW (Eds.), *Disease of muscle*, part II. (1st. Edn.), New York, North-Holland Publishing Co., PP: 259-285.

21- SAS Institute (1985). SAS Users Guide: statistics (Cary, SAS Institute). P: 378.

22- Sylven, C; Jansson, E and Book, K (1984). Myoglobin content in human skeletal muscle and myocardium: relation to fiber size and oxidative capacity. Cardiovasc. Res., 18: 443-446.