ACTA SCIENTIFIC NUTRITIONAL HEALTH

Volume 3 Issue 6 June 2019

Research Article

Caffeine Induces Changes in Hematological, Biochemical, and Cardiovascular Parameters in Quail

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Received: April 17, 2019; **Published:** May 29, 2019

Abstract

Caffeine is one of the most widely consumed pharmacological substances in the world, found in food and drinks. The effects of caffeine on human health and cardiovascular disease have been the focus of much debate, and much research has been performed in various animal models, including some in chicken. Previously, we reported caffeine to cause blood biochemistry changes and heart failure in young chickens (*Gallus gallus*). Recently, quails are increasingly used as a more economical and convenient bird model. Here, a trial was conducted to assess the suitability of adult quail as an avian model for caffeine research. Fifty six, 20-week-old laying Japanese quails (*Coturnix coturnix japonica*) were randomly divided into two groups (28 birds each with 7 replicates): one with no added caffeine, and one which was placed on a 15 mg/kg body weight/day caffeine in drinking water. On d 21, 14 birds per group were sacrificed and analysed for physiological and hematological parameters. Results showed that body weight, liver, heart and spleen relative weight were not affected by caffeine ($P > 0.05$), whereas a significant right ventricular hypertrophy was found ($P < 0.01$). Plasma alanine aminotransferase and aspartate aminotransferase activity were highly decreased in caffeine group ($P < 0.001$), pointing at a hepatoprotective effect. Hematocrit, plasma hemoglobin, total protein, albumin, globulin, glucose, triglyceride, cholesterol and low density lipoprotein (LDL) were significantly higher in caffeine group ($P < 0.001$). The hepatoprotective effect and the striking elevation of cholesterol and LDL by caffeine are consistent with those reported in humans, demonstrating the suitability of adult quail as an animal model for caffeine research.

Keywords: Avian Models; Blood Biochemistry; Caffeine; Liver Enzyme; Quail

Introduction

Domestic chicken are used as an attractive animal model for studies of a variety of diseases such as ovary cancer, pulmonary hypertension and diabetes, and for nutritional studies as appropriate omnivorous model [1]. More recently, quails attract attention as an alternative avian animal model because of the lower maintenance cost associated with its small body size (80-300 g) and with the short generation interval among birds [2].

Caffeine (1,3,7-trimethylxanthine) is a natural purine alkaloid found in coffee beans, tea leaves, cocoa beans, cola nuts and other plants. It is probably the most frequently ingested pharmacologically active substance in worldwide [3,4]. It has been well documented that caffeine has effects on the central nervous system and on hormonal, metabolic, muscular, cardiovascular, pulmonary, and renal functions [5]. Caffeine is completely metabolized to 1-methylxanthine and 1-methyluric acid from the paraxanthine intermediate. The primary site of caffeine metabolism is the liver, where the cytochrome P450 isoform CYP1A2 accounts for almost 95% of the primary metabolism [6].

Both the public and the scientific community have expressed concern about the potential for caffeine to produce adverse effects on human health, although most data were solely based on observational studies [7]. It is judged that in the absence of more robust data associated with low levels of administered caffeine, an upper intake of 2.5 mg kg-1 BW day-1 is an amount on which to base risk assessments of caffeine consumption in children. Moreover, doses of 5 to 8 mg/kg in adults, caused mild anxiety, respiratory stimulation, cardiovascular effects, excessive urinary output and increased gastric secretion [8].

There have been no investigations of the effects of caffeine in avian, apart from our previous studies showing that 12.5-100 mg/kg/BW dosage of caffeine can induce heart failure [9] and blood

parameters changes in young chicks [10]. In this paper we tried to establish whether or not quail could be a substitute for chicken and mammals in the research on the nutritional effects of caffeine.

Materials and Methods

Animals and housing

Fifty six, 20-week-old laying Japanese quails (*Coturnix coturnix japonica*) (mean body weight = 245 ± 5) were randomly divided into two groups (28 birds each with 7 replicates): a placebo (control), which remained on a normal diet (no added caffeine), and a caffeine group, which was placed on a 15 mg/kg body weight/day caffeine-added into water (which approximately is equivalent to 10 cups of coffee per day for 70 kg person). All procedures were approved by the Tarbiat Modares University Animal Care and Use Committee. The quails were obtained from a closed stock colony maintained at poultry farm of Tarbiat Modares University. Birds were kept in cages (4 birds in each) under constant conditions of illumination (16hr light: 8hr darkness) and temperature (25 ± 2 °C) and provided with food and water ad libitum for 3 weeks. Egg production was recorded weekly for each group.

Sample collection

On d 21 of experiment whole blood sample samples were collected by heart puncture from 2 birds per replicated cage (14 birds per group). Then, birds were killed and internal organs weighed. The hearts were weighed, and the atria, pericardium, major vessels and fat were trimmed off. The right ventricle (RV) and left ventricle (LV; including the ventricular septum) were separated, their individual weights were measured on an analytical balance; total ventricular (TV) weight measured and the RV/TV and RV/LV+ septum ratios were calculated as an index of RVH [11].

Hematological and biochemistry analysis

A portion of the blood sample was stored at 4°C pending hematocrit (HCT) and hemoglobin analysis, while the remainder was centrifuged and the plasma stored at -20°C for later analysis. Hematocrit percentage was determined in whole blood samples by centrifugation of microhematocrit capillary tubes at 15,500 × g for 5 min. Hemoglobin (Hb) concentration was determined according to the cyanmethemoglobin method using a commercial kit (Pars Azmun, Tehran, Iran) [10]. Plasma was prepared by centrifugation (1000 × g for 20 min) and stored at -20°C for analysis. Concentrations of total protein (TP), albumin (ALB), glucose, triglyceride (TG), cholesterol (CHL) and high density lipoprotein (HDL) were determined in plasma samples. Low density lipoprotein (LDL) was calculated by subtracting CHL from HDL. The analyses of the plasma samples were performed by spectrophotometric methods using commercially available kits (Pars Azmun, Tehran, Iran). Plasma creatinin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and gammaglutamyl transferase (GGT) activities were measured using commercial diagnostic kits (Pars Azmoon, Tehran, Iran).

Statistical analysis

A t-test using the general linear model procedure of SAS software (SAS, 2004) was used to estimate significant differences ($P < 0.05$). All data are presented as mean ± SE.

Results

Organ weight and blood parameters

No differences in egg production was observed between treatments (data not shown). Body weight and organs relative weight are shown in Table 1. Body weight, liver, heart and spleen relative weight were not affected by caffeine ($P > 0.05$). The RV/TV and RV/LV ratio was significantly increased in the caffeine group ($P<0.01$). Hematocrit and hemoglobin also significantly increased in the caffeine group ($P < 0.01$) (Table 2).

Group	Body weight (g)	Liver (%)	Spleen (%)	Heart (%)
Placebo (n=14)	246.6 ± 8.2	3.11 ± 0.16	0.073 ± 0.005	0.65 ± 0.03
Caffeine (n=14)	245.7 ± 6.4	4.08 ± 0.48	0.087 ± 0.013	0.72 ± 0.02
P-value	0.92	0.07	0.36	0.09

Table 1: Body weight, relative weight of heart, liver and spleen of quails at the end of the experiment (Mean ± SE).

Group	HCT (%)	Hb (g/dL)	RV/TV	RV/LV
Placebo (n=14)	25.48^{b} ± 0.99	6.52^{b} ± 0.15	0.192^{b} ± 0.004	0.23^{b} ± 0.006
Caffeine (n=14)	32.96^{a} ± 1.06	7.55^{a} ± 0.22	0.212^{a} ± 0.004	0.27^{a} ± 0.009
P-value	0.0001	0.0008	0.003	0.0059

Table 2. Hematocrit (HCT), hemoglobin (Hb), right ventricular to total ventricular ratio (RV/TV) and right ventricular to left ventricular + septum ratio (RV/LV) in quail (Mean + SE).

^{a,b} Values within a row with different superscripts differ significantly at $P<0.05$.

Plasma Biochemistry and enzyme activity

Effect of caffeine on liver enzymes of AST, ALT, GGT, LDH and creatinine in quail plasma are shown in Table 3. Even though creatinine, LDH and GGT level was not affected by caffeine ($P > 0.05$) but AST and ALT were remarkably decreased by caffeine supplementation ($P < 0.01$).

As shown in Table 4, the levels of quail plasma total protein, albumin, globulin, glucose, TG, cholesterol, HDL and LDL were remarkably higher in caffeine group vs placebo ($P < 0.001$).

Group	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	LDH (U/L)	Creatinine (IU/L)
Placebo (n=14)	14.85[a] ± 1.2	5.25[a] ± 1.1	13.6 ±2.7	249 ± 33	0.41 ± 0.08
Caffeine (n=14)	1.73[b] ± 0.2	2.45[b] ± 1.1	12.4 ± 1.3	188 ± 43	0.40 ± 0.10
P-value	0.0001	0.028	0.69	0.28	0.91

Table 3: Plasma liver enzymes of alanine-amino transferase (ALT), aspartate-amino transferase (AST), gammaglutamyl transferase (GGT), lactate dehydrogenase (LDH) and creatinine in quail (Mean + SE).

[a,b] Values within a row with different superscripts differ significantly at $P<0.05$.

Discussion

The present study was performed to establish the suitability of quails as a more convenient avian substitute for chicken as a model for caffeine research. To our knowledge, this is the first report of the effects of caffeine on adult quails. Our previous studies in chicken showed that using doses above 10 mg/kg BW caffeine has significant effects on performance and physiological parameters [9,10], and in the current study 15 mg/kg BW was tested. The effects observed on blood parameters in quail were different from those in broilers. The reason maybe that broilers are juveniles highly selected for high growth rates and therefore not representative for normal adults [10]. However, the results obtained here in adult quails were similar concerning the right ventricular hypertrophy [9]. Young broiler chicken in the growing period are highly prone to develop right ventricular hypertrophy [9], here we show that adult quail are susceptible too, albeit to a lesser extent. The mechanism behind the hypertrophy is not clear. A clue maybe taken from the increase in HCT and Hb, which can indicate hypoxia resulting in compensatory erythropoiesis [12].

Group	TP	ALB	GLB	TG	TC	HDL	LDL	Glucose	UA
Placebo (n=14)	4.40[b] ± 0.08	2.49[b] ± 0.03	1.90[b] ± 0.07	440[b] ± 10	179[b] ± 5	98[b] ± 1.0	80[b] ± 5	211[b] ± 8	4.5[b] ± 0.04
Caffeine (n=14)	5.58[a] ± 0.12	2.86[a] ± 0.04	2.72[a] ± 0.14	512[a] ± 7	255[a] ± 6	107[a] ± 0.9	147[a] ± 6	249[a] ± 4	5.3[a] ± 0.1
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0001	0.0006	0.002

Table 4: Blood biochemistry parameters at day 21 of the experiment (TP= Total protein (g/dL), ALB= Albumin (mg/dL), GLB= Globulin (mg/dL), TG= Triglyceride (mg/dL), TC= total cholesterol (mg/dL), HDL= High density lipoprotein (mg/dL), LDL= Low density lipoprotein (mg/dL), UA=Uric acid (mg/dL)) (Mean + SE). **±**

Caffeine may cause hypoxia in birds by increasing basal metabolic oxygen requirements [9], and the corresponding increases in hematocrit and cardiac output may result in increased pulmonary arterial pressure and cause both a volume and a pressure overload on the right ventricle [11]. Alternatively, the increase in HCT and Hb may result from caffeine induced diuresis [7,13] and resulting hemoconcentration. If the latter was the case, one could expect similar increases in total protein, albumin and other blood chemistry parameters (Table 4), which was indeed found. With the other parameters, such as creatinine, and liver enzymes (Table 3), the situation is less clear. Reports in the literature differ about the value of the latter as indicators of hemoconcentration, but at least one study showed that AST levels indeed did not change upon hemoconcentration [14].

We conclude that based on the most reliable indicators of hemoconcentration HCT, Hb, TP and ALB, hemoconcentration is the most likely cause and consistent with the diuretic effects of caffeine. The fact that the liver enzymes AST and ALT were even significantly lower as opposed to total protein, albumin and other blood chemistry parameters points clearly at a different mechanism, and could be interpreted as a positive effect of caffeine on liver function. This is consistent with the hepatoprotective effect of caffeine described in rats [15]. Also, caffeine comsumption is associated with lower prevalence of chronic liver disease and decreased plasma ALT, and AST levels human [16-18].

So except for the liver enzymes most of the values increased in the caffeine group were consistent with hemoconcentration, with an approximate increase ranging from 110-130%. Notable exceptions are GLB (143%), cholesterol (142%), and most strikingly LDL with 183%. We do not have an explanation for the increase in GLB, however, the elevated total cholesterol and LDL levels are consistent with the negative effects on cardiovascular health associated with high coffee consumption in humans [19].

Conclusion

As far as the authors know, this is the first report of the use of a caffeine on adult birds. As opposed to results obtained in young broilers, the present results show that adult birds exhibit more features associated with human use of caffeine. Caffeine in adult quail appeared to have a hepatoprotective effect, and caused dyslipidemia characterized by increased total cholesterol and LDL. This indicates that the adult Japanese quail is a suitable and cheap model for caffeine research.

Disclosure Statement

No conflict of interest was reported by the authors.

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