



PLASMA CHOLESTEROL AND TRIGLYCERIDE LEVELS IN BLACK-NECKED PHEASANTS, GRAY AND CHUKAR PARTRIDGES WITH CANNIBALISM TREATED WITH SILYMARIN AND TRYPTOPHAN

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Summary

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Plasma cholesterol and triglyceride levels were studied in black-necked pheasants, gray partridges and chukar partridges divided into 4 groups (n=12 in each group; n=6 by sex): first group (negative control) – clinically healthy birds, second group – birds treated with tryptophan, third group – birds treated with silymarin, fourth group (positive control) – birds with signs of cannibalism. In the pheasants and chukars with signs of cannibalism, cholesterol levels were higher than these in the control group. On the opposite, the triglyceride levels in the other three groups of birds with cannibalism were substantially lower than those in the healthy group. Supplementation with tryptophan tended to increase blood cholesterol and triglycerides in pheasants, gray and chukar partridges. In the groups treated with silymarin, the levels of the studied indicators were significantly higher in comparison to birds with cannibalism.

Key words: cannibalism, chukar partridge, game birds, gray partridge, triglycerides, pheasants, silymarin

INTRODUCTION

The commonest game birds in Europe and Asia, including in our country, are black-necked pheasants (*Phasianus colchicus*), gray partridges (*Perdix perdix*) and chukar partridges (*Alectoris chukar*). They belong to order Galliformes, family Phasianidae. When wild birds are reared in an artificial environment, they may develop a number of problems. In captivity,

they are not able to perform physiological behaviour due to limitations of the artificial environment (Birkl *et al.*, 2017; Daigle, 2017). Additionally, intensive husbandry often impairs vital parameters e.g. food, population density, lighting, temperature etc (Gilani *et al.*, 2013; Nicol *et al.*, 2013). For these reasons, birds manifest adverse behaviour that results in

diseases and significantly worsened welfare. Cannibalism is one of the major problems in game birds (Sedlackova *et al.*, 2004; Kjaer & Hocking, 2004; Rodenburg *et al.*, 2013). Blood biochemical assays are essential and widely used for diagnosis of various diseases in domestic birds, however data in game birds are few or insufficient (Suchy *et al.*, 2010; Vitula *et al.*, 2011; Hrabcakova *et al.*, 2014). The aim of the present study was to investigate the blood cholesterol and triglycerides in clinically healthy black-necked pheasants, gray partridges and chukar partridges, in birds manifesting cannibalism and to evaluate the possible prevention of this problem by supplementation with either tryptophan or silymarin with regard to their use as additional method for diagnosis of the condition. This study is a part from a large-scale research in the three game bird species.

MATERIALS AND METHODS

Each of the three bird species (black-necked pheasants, gray partridges and chukar partridges) was divided into 4 groups with 12 birds (6 male and 6 female) as followed: group I (negative control, n=12) included clinically healthy birds fed a balanced compound feed according to their age, produced by Topmix forage plant. Group II (experimental, n=12) comprised birds with signs of cannibalism, whose standard diet was supplemented with 21 g/kg of the amino acid tryptophan. Clinically diseased game birds from group III (experimental, n=12) were supplemented with 10 g/kg silymarin through the diet (78% *Extr. Silybum marianum*). Group IV (positive control, n=12) included untreated birds with clinical signs of cannibalism. The experiment lasted 30 days. By the end of the study

period, 1 mL blood samples were collected from each bird in heparinised tubes. Blood plasma was obtained after centrifugation at 3000 rpm. Plasma cholesterol and triglycerides were measured on an automated biochemical analyser (BS-120, Mindray, China). The experiment was conducted with permit No. 280 of the BFSa for wild game birds reared in captivity. Data were processed with IBM SPSS Statistics (SPSS-Inc., 2019, SPSS Reference Guide 26 SPSS, USA) using descriptive statistics and frequency distribution tables. Data from male and female birds were analysed separately, as sex may influence the studied parameters. All values were expressed as means \pm standard deviation. The level of statistical differences between groups was determined by the Tukey-Kramer test.

RESULTS

The effect of 30-day tryptophan supplementation on plasma cholesterol and triglycerides (TG) in black-necked pheasants (*Phasianus colchicus*) is illustrated in Table 1.

Plasma cholesterol in clinically healthy birds from group I was lower than values in birds treated with tryptophan (group II) and silymarin (group III) ($P < 0.001$). Silymarin-treated pheasants exhibited rather high blood cholesterol compared to those from groups I, II and IV ($P < 0.001$). Healthy male pheasants had substantially lower blood cholesterol than males treated with silymarin ($P < 0.001$). Cholesterol in clinically healthy females was statistically significantly lower than that in females treated with tryptophan ($P < 0.001$) and silymarin ($P < 0.001$). Cholesterol in male birds from group III exceeded significantly the values measured in groups II and IV ($P < 0.001$); the

Table 1. Blood cholesterol and triglyceride concentrations (mean \pm SD) in black-necked pheasants

Group	Sex	Cholesterol (mmol/L)	Triglycerides (mmol/L)
I	♂	2.94 \pm 0.45	2.12 \pm 0.83
	♀	2.69 \pm 0.45	3.15 \pm 1.47
	♂+♀	2.82 \pm 0.45	2.64 \pm 1.26
II	♂	4.11 \pm 0.80	1.23 \pm 0.44 ^{2b,3a}
	♀	4.92 \pm 0.88 ^{1b,2c,3c}	1.00 \pm 0.26 ^{2c,3b}
	♂+♀	4.52 \pm 0.91 ^{1a,2b,3c}	1.11 \pm 0.36 ^{2c,3c}
III	♂	6.68 \pm 1.18 ^{1c,2c,3c,4c,5a,6c}	2.63 \pm 0.82 ^{5a,6b}
	♀	6.72 \pm 1.37 ^{1c,2c,3c,4c,5a,6c}	2.10 \pm 0.80
	♂+♀	6.71 \pm 1.22 ^{1c,2c,3c,4c,5b,6c}	2.36 \pm 0.82 ^{5a,6b}
IV	♂	3.78 \pm 0.36 ^{7c,8c}	0.59 \pm 0.27 ^{1a,2c,3c,7b,8a,9c}
	♀	3.52 \pm 0.44 ^{7c,8c,9c}	0.90 \pm 0.22 ^{2c,3b,7a,8a,9a}
	♂+♀	3.65 \pm 0.41 ^{7c,8c,9c}	0.74 \pm 0.28 ^{1a,2c,3c,7c,9c}

^aP<0.05, ^bP<0.01, ^cP<0.001; 1 – vs Group I ♂, 2 – vs Group I ♀, 3 – vs Group I both sexes; 4 – vs Group II ♂, 5 – vs Group II ♀, 6 – vs Group II both sexes; 7 – vs Group III ♂, 8 – vs Group III ♀, 9 – vs Group III both sexes.

Table 2. Blood cholesterol and triglyceride concentrations (mean \pm SD) in gray partridges

Group	Sex	Cholesterol (mmol/L)	Triglycerides (mmol/L)
I	♂	3.42 \pm 1.05	2.13 \pm 0.22
	♀	3.37 \pm 1.36	2.25 \pm 1.03
	♂+♀	3.40 \pm 1.18	2.19 \pm 0.71
II	♂	3.81 \pm 0.49	1.54 \pm 0.32
	♀	4.22 \pm 0.46	1.29 \pm 0.14 ^{6a}
	♂+♀	4.00 \pm 0.50	1.42 \pm 0.28
III	♂	4.55 \pm 0.49	2.17 \pm 0.88
	♀	4.60 \pm 0.72	2.76 \pm 1.74
	♂+♀	4.58 \pm 0.59 ^{3b}	2.46 \pm 1.35
IV	♂	2.89 \pm 0.50 ^{7a,8b,9b}	1.31 \pm 0.31
	♀	2.90 \pm 0.26 ^{7a,8b,9b}	1.05 \pm 0.32 ^{8a,9a}
	♂+♀	2.90 \pm 0.38 ^{5a,6a,7b,8b,9c}	1.18 \pm 0.33 ^{8b,9b}

^aP<0.05, ^bP<0.01, ^cP<0.001; 3 – vs Group I both sexes; 5 – vs Group II ♀, 6 – vs Group II both sexes; 7 – vs Group III ♂, 8 – vs Group III ♀, 9 – vs Group III both sexes.

changes in female birds from group III vs group II and IV were similar (P<0.001).

Blood TG from group I were the highest compared to tryptophan-treated group (P<0.001) and vs untreated birds with

cannibalism (P<0.001). Higher TG values were found out in birds supplemented with silymarin compared to those that received tryptophan (P<0.01) and untreated birds from group IV (P<0.001).

Table 3. Blood cholesterol and triglyceride concentrations (mean ±SD) in chukar partridges

Group	Sex	Cholesterol (mmol/L)	Triglycerides (mmol/L)
I	♂	2.99±0.28	1.97±0.29
	♀	3.80±0.32	9.18±0.02 ^{1c}
	♂+♀	3.40±0.52	5.57±3.77 ^{1c,2c}
II	♂	4.50±0.60 ^{1b}	2.49±0.86 ^{2c,3b}
	♀	4.11±0.77	2.02±0.21 ^{2c,3c}
	♂+♀	4.30±0.69 ^{1b,3a}	2.26±0.64 ^{2c,3c}
III	♂	3.13±0.90 ^{4a,6a}	2.56±0.49 ^{2c,3b}
	♀	3.75±0.76	2.07±0.26 ^{2c,3c}
	♂+♀	3.44±0.86	2.31±0.45 ^{2c,3c}
IV	♂	4.05±0.44	1.61±0.33 ^{2c,3c}
	♀	4.06±0.72	1.79±0.56 ^{2c,3c}
	♂+♀	4.06±0.57	1.71±0.46 ^{2c,3c}

^aP<0.05, ^bP<0.01, ^cP<0.001; 1 – vs Group I ♂, 2 – vs Group I ♀, 3 – vs Group I both sexes; 4 – vs Group II ♂, 6 – vs Group II both sexes.

Male healthy pheasants had higher TG concentrations than diseased untreated males (P<0.05). TG in female healthy birds exceeded considerably the levels in group II (P<0.001) as well as in group IV (P<0.001). Male pheasants from group III had higher TG than those from group IV (P<0.01), as did silymarin-treated females vs untreated females (P<0.05).

By the end of the 30th day of supplementation, blood cholesterol in healthy gray partridges (*Perdix perdix*) was lower than those in birds treated with silymarin (P<0.01; Table 2). Cholesterol concentrations in birds from group IV were statistically significantly lower than in birds receiving both tryptophan (P<0.05) and silymarin (P<0.001). Male gray partridges from group III had higher cholesterol in blood than untreated males from group IV (P<0.05). Comparable changes were observed in females from group III vs group IV yet at a greater extent (P<0.01). Blood TG in gray partridges treated with silymarin (Table 2) were the highest compared to those with cannibalism from

group IV (P<0.01). Silymarin-treated females demonstrated higher TG levels than untreated females (P<0.05).

Table 3 presents data from the 30-day dietary supplementation of chukar partridges (*Alectoris chukar*) with tryptophan and silymarin.

Blood cholesterol was higher in chukar partridges that received tryptophan whereas values in the clinical healthy group I were the lowest (P<0.05). Male healthy chukar partridges had lower cholesterol concentrations than tryptophan-supplemented males (P<0.01) but higher levels than silymarin-treated male chukar partridges (P<0.05). Blood TG data showed that clinically healthy chukar partridges had the highest levels vs the group treated with tryptophan (P<0.001), silymarin (P<0.001) and untreated birds with cannibalism (P<0.001). In healthy females, TG concentrations were statistically significantly higher than those in all other male and female groups (P<0.001).

DISCUSSION

The presented results on blood cholesterol levels in healthy pheasants were similar to those published by other researchers (Suchy *et al.*, 2010; Hrabcakova *et al.*, 2014). The concentrations in healthy birds were however lower than in pheasants manifesting cannibalism. After treatment of pheasants with tryptophan, blood cholesterol increased substantially whereas the supplementation with silymarin led to almost twice higher levels.

In gray partridges, the observed tendencies in blood cholesterol in healthy birds were opposite to those in pheasants. Clinically diseased partridges had lower blood cholesterol than healthy birds (Vitula *et al.*, 2011). After the addition to tested dietary supplements, a moderate increase of blood cholesterol occurred in birds treated with tryptophan and almost twice higher concentrations – in birds treated with silymarin.

In chukar partridges, cholesterol of healthy birds was lower than values in birds with cannibalism, similarly to pheasants. Similar blood cholesterol concentrations in gray partridges were previously reported (Rodriguez *et al.*, 2004; Suchy *et al.*, 2010). After supplementation with tryptophan, blood cholesterol increased moderately similarly to what happened in the other two bird species. Conversely, the supplementation with silymarin in this game species resulted to reduction of blood cholesterol concentrations.

Significant species-specific differences in blood cholesterol concentrations were found out in pheasants, gray and chukar partridges (Suchy *et al.*, 2010). Increased cholesterol may be due to enhanced biosynthesis and weight gain (Lloyd & Gibson, 2006), as well as to egg laying and accumulation in the egg yolk (Suchy

et al., 2001; Hrabcakova *et al.*, 2014). It was proved that blood cholesterol was substantially increased during breeding and egg production in birds (Harr, 2002).

Similar data were reported about plasma TG levels in pheasants (Lloyd & Gibson, 2006). In healthy pheasants, TG was statistically significantly higher than in birds with cannibalism. It is affirmed that in diseased birds, blood TG decreased after periods of fasting (Kurima *et al.*, 1994). In our view, the increased level of stress also contributed to lower plasma TG concentrations in pheasants manifesting cannibalism. After the supplementation of diseased pheasants with tryptophan, their plasma TG levels were considerably increased. In birds treated with silymarin this increase was even more pronounced and TG concentrations in this group attained levels of healthy controls. With respect to sex, male pheasant responded with higher level of TG synthesis after treatment with both supplements, compared to females.

A similar trend was preserved in gray partridges, where plasma TG levels in diseased birds were significantly lower than those in healthy controls. After supplementation with tryptophan, TG levels increased whereas after treatment with silymarin, TG even exceeded the average concentrations in controls from the same species. The time course of plasma TG in chukar partridges was similar to those of the other two species. The diseased birds had lower TG concentrations than healthy ones, and TG increased following treatment with tryptophan and silymarin. It was reported that blood TG in adult game birds – pheasants and partridges – were higher than those in young birds from the respective species (Balasch *et al.*, 1973; Rico *et al.*, 1977). In this study, TG levels in healthy chukar partridges were much

more increased than in the other two species as a species-specific feature, in line with earlier reports (Rodriguez *et al.*, 2004; Lloyd & Gibson, 2006). The large differences in plasma cholesterol and TG among the game species may be due to circadian rhythms, feeding (Palomeque *et al.*, 1991; Villegas *et al.*, 2002; Harr, 2002), age and sex (Meluzzi *et al.*, 1992; Strakova *et al.*, 1993). In the present study, significant sex-related differences for blood cholesterol and TG concentrations were not observed.

CONCLUSIONS

In the pheasants and chukars with signs of cannibalism, cholesterol levels were higher than these in the healthy controls. Cannibalism in pheasants, gray and chukar partridges was characterised with lower blood triglyceride levels compared to the healthy group.

Supplementation with the tested substances (tryptophan and silymarin) improved the health of game birds manifesting cannibalism and the doses achieved the desired effect. The dietary supplementation with tryptophan tended to increase moderately blood cholesterol and triglycerides in pheasants, gray and chukar partridges. In the groups treated with silymarin, the levels of the studied indicators were significantly higher compared to untreated birds with cannibalism.

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