

REGULAR ARTICLE

Effect of silymarin supplementation on some productive and hematological parameters in meat type male Japanese quails

Hristo Lukanov¹, Ivelina Pavlova^{2*}, Veselin Ivanov³, Todor Slavov¹, Yoana Petrova², Nadya Bozakova²

¹Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria, ²Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria,

³Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

A total of 90 14-day-old meat type male Japanese quails were included in the experiment and equally divided into 3 groups: control group (CG) without treatment, experimental group 1 (EG 0.5%), supplemented with silymarin 0.5% and experimental group 2 (EG 1%), supplemented with silymarin 1%. The aim of the current study was to establish the effect of supplementation with different doses of silymarin extract on the growth performance and some hematological parameters in male Japanese quails. Main growth performance (body weight, feed consumption and weight gain) were examined and FCR was calculated. Some hematological (WBC, RBC, HGB and HTC) and biochemical (triglycerides, AST and ALT) parameters were determined. The highest average body weight was observed in EG 0.5% group at 35-day-old age and the lowest in EG 1%, 239.61 ± 2.96 g and 231.74 ± 2.96 g respectively. The highest values of the weight gain were calculated in EG 0.5% group, 67.02 ± 2.03 g during the first week of fattening and the lowest was observed in EG 1% group at the fifth week after hatching, 23.30 ± 2.03 g respectively. The lowest values of FCR were observed during the first week of the trial, 2.42 ± 0.08 kg/kg to 2.61 ± 0.13 kg/kg in EG 1% and the control group respectively. The supplementation of 0.5% and 1% silymarin did not significantly change the studied hematological and biochemical parameters. A tendency of higher values of triglycerides and lower values of AST and ALT was observed in the groups, supplemented with silymarin compared to the controls. From the obtained results of the present study we can conclude that the silymarin supplementation at higher doses (0.5% and 1%) did not have effect on the main growth performance and some hematological and biochemical parameters of male Japanese quails.

Key words: Silymarin; Japanese quail; Blood profile; ALT; AST

INTRODUCTION

Silymarin is a flavonoid which is a part of benzo gamma-pyrone (Hemat, 2004). It is obtained from the seeds of milk thistle (*Silybum marianum*) through extraction (Kroll et al., 2007). The main active components of silymarin are silybinin, silydianin and silycristin (Kabel, 2014), isosilybin, isosilycristin and taxifolin were also reported (Kroll et al., 2007). Silybinin has the most significant cytoprotective effect (Karimi et al., 2011) and mainly hepatoprotective properties (Vargas-Mendoza et al., 2014). It is often used in the prevention and treatment of a number of liver diseases (Polyak et al., 2010). Previous studies established the hepatoprotective role of silymarin in experimentally induced intoxications and concluded that it might be used in birds to prevent the effects of Aflatoxin B1 and cadmium

intoxication (Karvanmoghadam, 2014; Makki, et al., 2014; Tahir et al., 2017). Furthermore, silymarin has very good radioprotector properties (Suchý et al., 2008), it could be used as oxidative antistresor (Behboodi et al., 2017), also in prevention and treatment of cancers, renal and pancreas protection, etc (Karimi et al., 2011).

Silymarin is used as therapeutic agent mostly in human medicine. In veterinary medicine it has relatively limited application based on its use for pets liver diseases. It is not implemented for treatment of liver diseases and intoxications in productive animals although there are a number of studies in this regard. The most risky sectors in livestock breeding, where the liver function might be damaged, are dairy livestock, egg poultry and fattening. There is a lack of information about silymarin supplementation in healthy

*Correspondence author:

Ivelina Pavlova, PhD - Department of "Pharmacology, Veterinary Physiology and Physiological Chemistry", Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria, E-mail: ivelpp@abv.bg, Tel: +359/42 699623

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animals. From the previous studies about the effect of silymarin on the productivity of farm animals can be concluded that it is favorably influenced (Tedesco, 2001; Quarantelli et al., 2004; Tedesco et al., 2004; Righi et al., 2005; Quarantelli et al., 2009; Abou-Shehema et al., 2016). The studied concentrations of silymarin supplemented in the feed of healthy and intoxicated animals were mostly less than 0.1%. Relatively few studies were based on higher doses of silymarin administered orally with the feed (Abou-Shehema et al., 2016; Makki et al., 2013; Kalantar et al., 2014).

Japanese quail (*Coturnix japonica*) is appropriate species for experimental work because of its rapid growth, short generation interval and high reproduction rate and fertility. It is also included in the intensive production of meat and eggs (Genchev, 2014).

There is a lack of information in the literature about silymarin supplementation at higher concentrations in healthy birds and its effect on growth performance and some main hematological parameters including biochemical indicators about the liver function. Therefore the aim of this study was to investigate the effect of silymarin supplementation on the growth performance and some hematological parameters in male meat type Japanese quails.

MATERIALS AND METHODS

Herbal extract

Silymarin - Milk Thistle Extract Powder, Wuxi Gorunjie Technology Co., LTD, Batch Number: GRJ-Sil-20070620 was used as feed supplement.

Animals and husbandry

A total of 90 14-day-old male Japanese quails were included in the experiment. They were from a heavy line with wild plumage color, selected in the Poultry Unit of Faculty of Agriculture, Trakia University, Bulgaria. They were placed in cages in the animal house of Poultry Unit according to the species requirements and were equally divided into 3 groups: control group (CG) without treatment, experimental group 1 (EG 0.5%), treated with silymarin 0.5% and experimental group 2 (EG 1%), treated with silymarin 1%. Water and food were supplied *ad libitum*. The content of feed mixtures is given in Table 1. The quails were healthy and any signs of disease were not observed during the trial. Milk thistle extract was administered via feed and started fourteen days after hatching for 20 days at a dose rate of 5 g/kg (0.5%) and 10 g/kg feed (1%). Feed consumption was daily registered and the body weight of the quails was followed at four time intervals. Growth and FCR were calculated. Blood samples for biochemical and hematological analysis were collected from all groups on

Table 1: Nutrition specification of quail feed, used in the experiment

Parameters	Grower feed 14-28 days after hatching	Finisher feed 28-35 days after hatching
Metabolizable energy (ME), kcal/kg	2920	2900
Crude protein, %	22.4	19
Crude fibre, %	4.63	4.76
Lysine, %	1.38	1.12
Methionine, %	0.48	0.42
Threonine, %	0.81	0.6
Ca, %	0.97	1.02
P, %	0.43	0.40

the 35th day of age by using the basilic vein - *Vena cutanea ulnaris superficialis*.

Serum biochemical parameters

The selected biochemical parameters were measured by Semi-auto Chemistry analyzer BA-88 Mindray, China. Serum alanine aminotransferase (ALT, IU/L) was determined by commercial test kit (Chema Diagnostics ALT FLIFCC; REF-GPF245CH LOT-TY-222). Similarly, serum aspartate aminotransferase (AST, IU/L) was determined by commercial test kit (Chema Diagnostics AST FLIFCC; REF-GOF245CH LOT-TY-168). Triglycerides (TG, mg/dL) were measured using commercial test kit (Giesse Diagnostics REF 0075 LOT 6151).

Blood profile analysis

The blood samples were transported in cooler to the licensed laboratory of "National center for professional education and competence "America for Bulgaria", Trakia University, Stara Zagora. The hematological parameters were measured by automatic hematology analyzer „BC-2800VET” and automatic clinical chemistry analyzer „BS-120”. The number of red blood cells (RBC, $10^{12}/l$), white blood cells (WBC, $10^9/l$), hemoglobin (HGB, g/l) and hematocrit (HTC, %) were analyzed.

Statistical analysis

All data were analyzed by Statistica 13.0 software (Statistica for Windows; Stat – Soft, 2015). Mean (x), standard error of mean (SEM) and coefficient of variation (CV, %) values were calculated for each group. The differences considered statistically significant at $p < 0.05$, using Student's t-test, if the data were normally distributed.

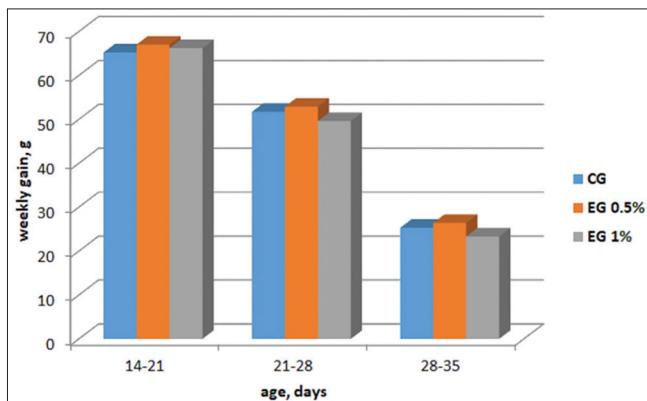
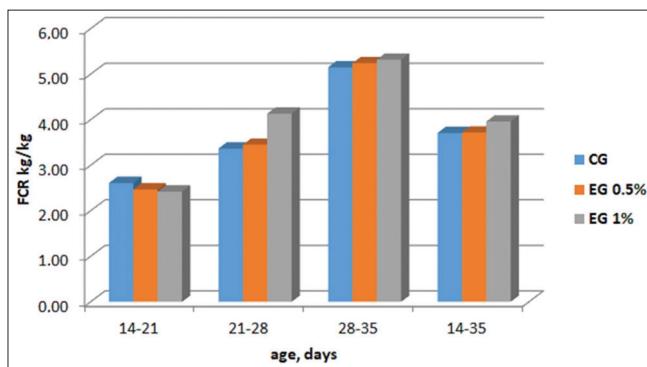
RESULTS

Body weight of the quails from the three groups at 2, 3, 4 and 5 weeks of age are presented in Table 2. The highest values of body weight during the whole fattening period were observed in EG 0.5% group. The differences between the groups were not statistically significant ($p > 0.05$).

Table 2: Body weight at 14, 21, 28 and 35 days of age

Age, days	Group		
	CG	EG 0.5%	EG 1%
14			
x	92.98	93.28	92.60
SEM	1.19	1.28	1.49
CV, %	4.25	4.56	5.34
21			
x	158.21*	160.29	163.66*
SEM	1.98	1.84	1.60
CV, %	4.15	3.82	3.24
28			
x	209.88	213.16	208.36
SEM	4.31	3.63	2.05
CV, %	6.81	5.64	3.26
35			
x	235.24	239.61	231.74
SEM	3.16	2.96	2.96
CV, %	4.45	4.10	4.23

* p<0.05

**Fig 1.** Average weekly gain in the control group (CG), experimental group 1 (EG 0.5%) and experimental group 2 (EG 1%) during the trial.**Fig 2.** Feed conversion ratio in the different age groups and for the whole period of fattening.

Variations between CG, EG 0.5% and EG 1% were not high, from 3.24 to 6.81% for the whole experimental period. During this period the quails from the three groups increased their body weight approximately 2.5 times. 35 days after hatching is the age for reaching slaughter

maturity in this meat type Japanese quails. At the beginning of the experiment the average body weight of the quails from the three groups was almost equal, varying from 92.60 ± 1.49 g in EG 1% to 93.28 ± 1.28 g in EG 0.5%. At 35 days of age the highest average body weight was measured in group EG 0.5%, and the lowest – in EG 1%, 239.61 ± 2.96 g and 231.74 ± 2.96 g respectively. The only statistically significant difference was found between CG and EG 1% at 21 days of age ($p < 0.05$). The three groups showed optimal values of body weight which is specific for the concrete meat line and age. Statistically significant effect of silymarin supplementation at concentration of 0.5% and 1% on the body weight was not found.

The changes in the weight gain during the three weeks of fattening in the control group, EG 0.5% and EG 1% are presented in Figure 1. The values of weight gain were progressively decreased with increasing age which is in line with approaching sexual maturity and reducing growth intensity. A tendency to higher weekly gain was observed in the group supplemented with 0.5% silymarin versus the control group. These changes varied from 1.22 to 11.7% in the different age categories and were not statistically significant. The highest weight gain was calculated in EG 0.5% group from 67.02 ± 2.03 g during the first week of fattening. The lowest weight gain was measured in group EG 1% during the fifth week after hatching, 23.30 ± 2.03 g respectively. The highest variations of this parameter were observed during the last week of the trial which is in conjunction with individual differences in the sexual maturity of the birds.

From the obtained data, presented in Fig. 2 we can conclude that the lowest values of feed conversion ratio (FCR) were observed during the first week of the trial (14-21-days of age), from 2.42 ± 0.08 kg/kg in group EG 1% to 2.61 ± 0.13 kg/kg in the control group. During the last week of fattening (28-35 days of age) the most inefficient use of the feed is considered, with values of FCR of 5.32 ± 0.23 kg/kg in group EG 1%, and 5.15 ± 0.47 kg/kg in group CG. The only statistically significant differences between the groups were during the second week of the experiment ($p < 0.05$).

The results from the biochemical analysis of the blood samples on 35 days of age are presented in Table 3. The values of triglycerides were significantly higher in group EG 0.5% compared to CG. A tendency to decreasing the values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was observed in the groups supplemented with silymarin versus the control quails.

The values of the studied hematological parameters (number of red blood cells, white blood cells, hemoglobin

Table 3: Biochemical indicators for the liver function (TG, AST and ALT)

Group	Indicator		
	TG (mg/dL)	AST (U/L)	ALT (U/L)
CG			
x	13.95*	454.33	44.0
SEM	1.22	44.66	6.38
CV, %	19.57	21.98	32.43
EG 0.5%			
x	16.70*	386.67	36.67
SEM	0.55	36.33	4.12
CV, %	7.40	21.01	25.09
EG 1%			
x	14.65	408.50	36.00
SEM	1.85	32.19	5.48
CV, %	28.21	17.62	34.02

TG – Triglycerides; AST - aspartate aminotransferase; ALT - alanine aminotransferase

Table 4: Hematological parameters (number of white blood cells, number of red blood cells, hemoglobin and hematocrit) of the birds from the control and experimental groups.

Group	Indicator			
	WBC, 10 ⁹ /l	RBC, 10 ¹² /l	HGB, g/l	HTC, %
CG				
x	25.92	2.70	160.17	37.45
SEM	0.40	0.16	8.33	1.82
CV, %	3.46	13.57	11.63	10.85
EG 0.5%				
x	26.33	2.69	161.67	36.90
SEM	0.55	0.07	4.81	1.02
CV, %	4.65	6.00	6.65	6.16
EG 1%				
x	26.38	2.68	159.40	37.30
SEM	0.41	0.14	5.59	1.21
CV, %	3.07	10.78	7.01	6.47

WBC - White Blood Cell Count; RBC – Red Blood Cell Count;
HGB - Hemoglobin; HTC - Hematocrit

and hematocrit) for the control and experimental groups on 35 days of age are presented in Table 4. The differences between the groups were not statistically different which showed lack of effect of silymarin supplementation in concentration 0.5% and 1% in the feed on the studied hematological parameters.

DISCUSSION

In the current study we established characteristic age dynamics in the changes of the body weight in meat type male Japanese quails. The results from a number of previous studies were in line with ours (Milvielle, 2002; Genchev, 2014; Karthika and Chandrasekaran, 2016). Body weight of the quails from the three groups at 35 days of age was significantly higher than the presented from Wilkanowska and Kokoszynski (2011) in Pharaoh quails at 42 days of age (169.1 g). Following the age development of this parameter

in the three studied groups, we can conclude that a tendency to higher values of the body weight was observed in the birds supplemented with 0.5% silymarin in the feed compared to controls and EG 1%. In another experiment about the effect of 0.5% silymarin on the body weight in Bandarah chickens, Abou-Shehema et al. (2016) obtained similar results. Makki et al. (2013) reported that the highest body weight was measured in 5-week-old non-intoxicated broiler chickens supplemented with 0.5% silymarin followed by the group supplemented with 1% silymarin in the feed. Schiavone et al. (2007) did not find significant effect of supplementation of lower doses of silymarin (40 and 80 ppm) on the growth performance in broilers. We observed similar results about the average weekly gain. A tendency to increasing the values of the weight gain was observed in the last two weeks of the fattening period in the EG 0.5% compared to the other two groups. On the contrary, Kalantar et al. (2014) reported negative impact of 0.5% silymarin supplementation in the feed of broiler chickens on the body weight gain versus the control group.

Feed conversion ratio is one of the most important economic features that characterize the efficiency of the feed utilization and its transformation into unit of production. It binds two important parameters – weight gain and feed consumption. In our study we found the most ineffective utilization of the feed in EG 1%. These results can be explained with the negative effect of the high doses of silymarin on gastrointestinal tract (Anonymous, 1999; Jacobs et al., 2002). The chosen doses of silymarin were not toxic so apparent disturbances of the digestive system were not observed. On the other hand the higher values of FCR can be explained with the higher consumption of the feed in groups EG 0.5% and EG 1%. Makki et al. (2013) reported similar results – with increasing the concentration of silymarin from 0 to 0.5% and 1%, higher consumption of feed was observed in non-intoxicated broilers. In previous study with broiler chickens Kalantar et al. (2014) found more effective utilization of feed in the group supplemented with 0.5% silymarin compared to control group. We did not find such positive effect of silymarin supplementation in concentration 0.5%.

In our study three serum biochemical parameters were examined (triglycerides, AST and ALT) to evaluate if there is any influence of silymarin extract supplementation in 0.5 and 1% dose on the liver function. Our results showed statistically significant increase in the values of triglycerides in the 0.5% supplemented silymarin group (EG 0.5%) in comparison to control group ($p < 0.05$). The other experimental group (EG 1%) showed nonsignificant increase in the triglycerides values in comparison to CG.

In previous study, a slight, but not significant, increase in triglyceride levels was observed in silymarin supplemented groups of broiler chickens versus the controls, which is in line with our results (Schiavone et al., 2007). This probably indicate an increase in metabolism, a reduction of hepatic storage of lipids or increased lipid mobilization. Scholz et al. (2009) studied the reference values of a number of biochemical parameters including triglycerides, ALT and AST in male and female mature quails. Comparing their data with the results obtained from us it can be said that the values of triglycerides in the control and experimental groups were in the lower reference range for the species. Our average and minimal-maximal values of AST were similar to these obtained from Scholz et al. (2009) in Japanese quails and from Gylstorff and Grimm (1998) in doves. Unlike cited studies and our results Nazifi and Asasi (2001), Hamidipour et al. (2016), Sokól et al. (2015), Panda et al. (2017) and Abang et al. (2017) found significantly lower values of AST in Japanese quails. Such lower values of AST were reported from Albokhadaim et al. (2013) in local chickens from Saudi Arabia and Abou-Shehema et al. (2016) in Bandarah chickens. Regarding the data for the other liver enzyme ALT, it can be concluded that our results were higher than the reference range for Japanese quails, reported by Scholz et al., 2009. Similar results were found from Sokól et al. (2015) in quails with experimental coccidiosis treated with toltrazuril. The values of ALT reported by Mnisi et al. (2017) in 9 week old male Japanese quails, Hamidipour et al. (2016) and Panda et al., (2017) in the same bird species were in line with our results. These differences can be explained with the different productive types of the experimental birds and the used laboratory equipment, diagnostics and methodology. We found that the values of the serum enzymes AST and ALT were non-significantly decreased after 0.5 and 1% silymarin supplementation in comparison to control group (CG). Similarly to our results, Tahir et al. (2017) found lower values of AST and ALT after 30 days supplementation of silymarin in Japanese quails. Abou-Shehema et al. (2016) reported similar results after addition of silymarin in the feed of Bandarah chickens. Suchý et al. (2008) verified the preventive effect of *Silybum marianum* seed cakes in the case of chlortetracycline therapy and measured hepatoprotective effect of silymarin. The AST and ALT activity in blood plasma of the experimental groups of broiler chickens supplemented with *Silybum marianum* seed cakes was lower compared to the control groups. Knowing that AST activity was found to be the most sensitive indicator of liver damage (Lumeij, 1997), we can conclude that the liver function was not affected during the experimental fattening process.

Silymarin supplementation (0.5 and 1%) did not change the values of the studied hematological parameters (WBC,

RBC, HGB and HTC) compared to the control group. These results are logical due to the fact that silymarin does not have an effect on hematopoiesis and related signs. The obtained results for the number of white blood cells (WBC) are in line with the results from other authors (Pravda et al., 1996; Nazifi and Asasi, 2001; Sujata et al., 2014) in groups including clinically healthy Japanese quails without treatment. The values of the number of red blood cells from our study correspond with the reference values for this species. Lower levels of this parameter in 5 week old quails were reported by Sujata et al. (2014). The average values for hemoglobin (HGB) obtained from us are higher than the results reported by Pravda et al. (1996), Nazifi and Asasi (2001) and Ayub Ali et al. (2012) in Japanese quails and significantly higher than the values cited from Sujata et al. (2014). The average values of the hematocrit (HTC) from the current study were in line with the presented from other authors in quails on the same age (Ayub Ali et al., 2012; Sujata et al., 2014).

CONCLUSIONS

The results of the present study suggest that supplementation of 1% silymarin in the feed of male Japanese quails worsen their fattening characteristics while 0.5% silymarin shows a positive impact.

A tendency to lower values of the liver enzymes (ALT and AST) was observed in the groups supplemented with silymarin extract compared to the nontreated birds.

Silymarin supplementation with the feed in concentration 0.5 and 1% in fattened quails did not show changes in hematological parameters WBC, RBC, HGB and HTC.

Due to the high price of the product and the impossibility of productive results compensation, the authors do not consider reasonable higher doses silymarin supplementation in the feed of fattened Japanese quails.

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