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SIMULTANEOUS ADMINISTRATION OF SILYMARIN AND DOXYCYCLINE IN JAPANESE QUAILS SUGGESTS PROBABLE HERB-DRUG INTERACTION

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Abstract

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The present study aimed to evaluate probable herb-drug interaction between silymarin extract and doxycycline and their effect on some biochemical serum parameters in Japanese quails. 120 14-day-old male Japanese quails were equally divided into 4 groups: control group without treatment (n = 30), group, treated with doxycycline (at a dose 10 $mg.kg^{-1}$ bw, via drinking water for four days, started 30 days after hatching, n = 30), third group, treated with doxycycline and silymarin 0.5% (started 14 days after hatching, via feed, for twenty days, n = 30) and group, treated with doxycycline and silymarin 1% as described above (n = 30). Water and food were supplied *ad libitum.* Blood samples were collected on 2, 4, 6, 9, 12, 24, 98, 100 and 102 h from the beginning of the treatment. The feed consumption and the body weight of the quails were registered. Growth and FCR were calculated. Silymarin supplementation provoked a tendency to slightly decrease in serum concentrations of the antibiotic with the time of the treatment compared to doxycycline only treated quails, suggested probable herb-drug interaction. Silymarin administration did not lead to better feed conversion ratio (FCR). The values of triglycerides and ASAT were significantly changed in doxycycline treated groups vs controls.

Key words: silymarin; doxycycline; herb-drug interaction; quail

Introduction

Raising Japanese quails is becoming a popular and gainful business in many countries. Quail takes an important place in poultry farming and is preferred species for scientific research because it has high reproduction rate and fertility, short reproduction period, highfarming per unit area and high feed conversion ratio. Bacterial diseases are the most common and destructive diseases of these birds (Glisson, 1998; Swayne et al., 2013). So, different types of antibiotics were used to prevent bacterial diseases, cure animals and birds, or as a feed additive to promote growth in some parts of the world (Swayne et al., 2013; Tavakkoli and Gooshki, 2014).

Doxycycline is one of the widely used antibacterial substances in poultry. It is a semisynthetic second generation tetracycline with some advantages over other antibiotics in this group: higher bioavailability after oral administration, lower affinity to Ca+ ions, better penetration in tissues and longer half-life of elimination (Papich and Riviere, 2013). These advantages, taken together with the lower MIC values against many Gram-positive and Gram-negative pathogens

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make doxycycline preferred antibiotic for therapy of bacterial diseases in veterinary medicine.

The ban of the use of growth-promoting antibiotics in animal feed in EU has forced investigators to research alternatives for increasing the body weight and improving the disease resistance in farm animals. These alternatives are of a great interest to the poultry industry. Quails in productive farms are exposed to a multitude of long- and short-acting stressors (e.g. heat stress, immune challenges, transport) which can alter their oxidant/antioxidant balance, leading to oxidative stress (Sies, 1991). Moreover, toxic substances in feed and the rapid growth rate of modern broiler strains can lead to significant metabolic and oxidative stress which can affect health and growth performance of broiler chickens, respectively quails (Carreras et al., 2004). Seeds of Milk Thistle are often supplemented in the feed of poultry as a growth stimulator, antioxidant and liver protector from toxins. The active extract of Milk Thistle is a mixture of flavonolignans, containing approximately 70-80% of silymarin and 20-30% chemically undefined fraction (Flora et al., 1998; Skottova et al., 2003). Basic component of silymarin which is thought to be responsible for its biological activity is silybinin (Ding et al., 2001).

Despite its use in poultry farming, limited information is available on the safety, interactions with other drugs or the mechanisms of interactions of silymarin. There are a number of investigations about the drug-drug interactions of silymarin in humans (Doehmer et al., 2008; Chang et al., 2009; Wu et al., 2009) but there is a lack of information about its pharmacokinetic interactions in quails. Therefore, the aim of this study was to investigate the effect of silymarin extract on doxycycline serum concentrations after their simultaneous administration in Japanese quails and to evaluate probable herb-drug interaction.

Material and Methods

Herbal extract

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

Drugs

Doxycycline hyclate, Doxy 200 ws, Interchemie Holland, Exp. Date: 11.2017; Batch No.: 256819 was used for treatment of the birds.

Doxycycline hyclate ≥ 98% (TLC) Lot# BCBF9827V (Sigma) and Oxytetracycline hydrochloride ≥ 95% (HPLC grade) Lot# BCBG9599V (Sigma) were used as internal standards during the HPLC analysis.

Animals and husbandry

A total of 120 14-day-old male Japanese quails were included in the experiment. They were placed in cages in the animal house of Poultry Unit of Faculty of Agriculture, Trakia University according to the species requirements and were equally divided into 4 groups: control group without treatment, group, treated with doxycycline, third group, treated with doxycycline and silymarin 0.5% and group, treated with doxycycline and silymarin 1%. Water and food were supplied *ad libitum.* The content of feed mixtures is given in Table 1.

Table 1

Nutrition specification of quail feed, used in the experiment

Ingredients	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 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 $g.kg^{-1}$ (0.5%) and 10 $g.kg^{-1}$ feed (1%). The treatment with doxycycline started 30 days after hatching via drinking water and lasted for 4 consecutive days at a dose of 10 $mg.kg^{-1}$ body weight. Each day, the solutions in drinking water were freshly prepared between 7.30 and 8 h in the morning and between 16 and 17 h in the afternoon. The 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 (each of 0.8 ml) from the groups, treated with doxycycline were collected on 2, 4, 6, 9, 12, 24, 98 and 100 h from the beginning of the treatment for analysis of serum concentrations of doxycycline. Blood samples for biochemical analysis were collected on 102 h from the beginning of the treatment from the controls, doxycycline and doxycycline + silymarin 1% treated groups. Serum was separated after centrifugation of blood samples at 1800 x g for 15 min and was stored at -20^{o}C until analysis.

Drug analysis

Doxycycline concentrations were analyzed by high-performance liquid chromatograph (HPLC) coupled with PDA detector (Baert et al., 2000). Shortly, 15 μL of the internal standard (11 μg/mL oxytertacycline) and 19.5 μL trifluoroacetic acid were added to 150 μL of serum samples. After vortexing, the samples were centrifuged for 10 min at 10800×g at 22 °C. The supernatants were transfered to HPLC vials and 20 μL were injected into the HPLC system (Thermo Fisher Scientific Inc., USA) (Laczay et al., 2001). The standard solutions of doxycycline were prepared in serum from untreated animals at concentrations of 10, 5, 2.5 and 1 $\mu g.mL^{-1}$. They were processed according to the described procedure.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated with Phoenix 6.0 software (Pharsight Corporation, Mountain View, CA, USA) using non-compartmental analysis after naive pooling of serum drug concentrations. Pharmacokinetic parameters were estimated with sparse sampling option. Naive pooling was done by using all individual data. Pharmacokinetic parameters for serum were first calculated on the basis of mean serum concentrations for each sampling time.

Serum biochemical parameters

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

Statistical analysis

Data were statistically evaluated using Statistica 6.0.

Results

The differences in average serum levels of doxycycline (C_{avg}) were statistically significant only between doxycycline treated group and doxy+silymarin 0.5% treated quails. A tendency to slightly decrease in serum concentrations of doxycycline with the time of the treatment was observed in groups treated with silymarin in comparison to doxycycline only treated quails (Figure 1.). The value of C_{max} was higher in doxy+silymarin 1% treated group in comparison to doxycycline only and doxy+silymarin 0.5% treated groups. The value of C_{min} was the lowest again in doxy+silymarin 1% treated group. The fluctuations in doxycycline treated quails were lower versus the groups administered with silymarin (Table 2.).

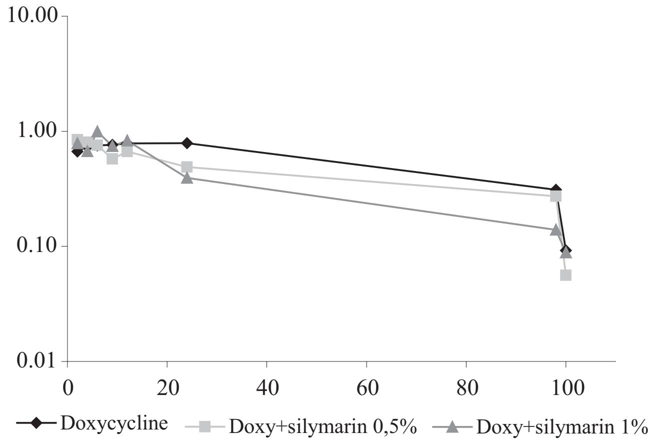

Fig. 1. Serum concentrations of doxycycline in male Japanese quails after oral treatment for four consecutive days at a dose of 10 mg.kg^{-1} bw administered with or without silymarin extract

Table 2

Pharmacokinetic parameters of doxycycline in male Japanese quails after oral treatment for four consecutive days at a dose of 10 mg.kg^{-1} bw administered with or without silymarin extract. The CV values are for C_{avg}. Level of significance: *p < 0.05

NCA	Unit	Doxycycline	Doxy+silymarin 0.5%	Doxy+silymarin 1%
Non-compartmental analysis, p.o.				
T_{max}	h	24	2	6
C_{max}	$\mu g.mL^{-1}$	0.788	0.843	0.994
T_{min}	h	2	24	24
C_{min}	$\mu g.mL^{-1}$	0.668	0.488	0.3935
C_{avg}	$\mu g.mL^{-1}$	0.760±0.05*	0.638±0.04*	0.699±0.05
Fluctuation%	%	15.780	55.70	85.88
CV	%	5.91	20.04	27.00

There were no statistically significant changes in the values of feed conversion ratio (FCR) between the four groups of quails (Table 3.). Silymarin administration did not lead to better feed convertion ratio. Similarly, the treatment with doxycycline with or without silymarin administration did not lead to statistically different values of the body weight (237.41±3.06; 232.07±2.91; 235.89±3.30 and 234.53±2.31 g for controls, doxycycline treated group, doxy+silymarin 0.5% and doxy+silymarin 1% treated group respectively).

Significantly higher values of Triglycerides in the groups treated with doxycycline and doxy+silymarin 1% versus the controls were observed (Table 4.). The values of ASAT were significantly lower in doxycycline treated quails in comparison to control group. A tendency to slightly decrease in the values of ALAT in silymarin administered quails versus the doxycycline treated and control groups was found.

Discussion

In recent years, antibiotics have been widely used in veterinary medicine to prevent and treat bacterial diseases. Because of the increasing antimicrobial resistance, the prudent use of antibiotics becomes more important and scientists are searching alternative ways to improve the effectiveness of antimicrobial therapy and reduce selection of antimicrobial resistance. Simultaneous use of drugs, herb extracts, probiotics and other feed additives may cause changes in absorption, disposition and metabolism of antibiotics which may alter serum and tissue concentrations, respectively effectiveness of the therapy. In previous study we examined the effect of probiotics on doxycycline disposition in gastro-intestinal tract of broiler chickens and concluded that the supplementation with *Lactobacillus* probiotics (*L. brevis* 51, *L. plantarum* 11 and *L. bulgaricus* 13) provoked no statistically significant changes in serum concentrations of doxycycline (Pavlova, 2015). The effect of doxycycline, administered with or without *Lactobacillus* probiotics, on mRNA expression of peptide transporter 1 (PepT1), liver expressed antimicrobial protein (LEAP-2) and ABC efflux transporters was evaluated and suggested a synergistic probiotics-doxycycline interaction in broiler chickens and probable beneficial effect on limitation of the absorption of toxins and improvement of efflux of xenobiotics (Milanova et al., 2016; Pavlova and Milanova, 2017). It is well known that flavonoids like silymarin may interfere with the metabolism of drugs by induction or inhibition of cytochromes P450 (Dai et al., 1997; Rajnarayana et al., 2004; Wu et al., 2009).

Therefore the present study was designed to evaluate the possible herb-drug interaction between silymarin extract and doxycycline in Japanese quails and their effect on some biochemical serum parameters showing liver health or damage. Our results showed that there is a tendency to slightly decrease in serum concentrations of doxycycline with the time of the treatment in groups treated with silymarin in comparison to doxycycline only treated quails (Figure 1.). In agreement with our results, a clinical study revealed that repeated administrations of silymarin may induce both intestine P-glycoprotein and CYP3A4 with consequent increase of the clearance and a concomitant decrease of C_{max} of metronidazole in humans (Rajnarayana et al., 2004). Chang et

Table 3

Feed conversion ratio in kg.kg^{-1} (FCR) in 35 day-old Japanese quails

	1			2			3			4		
	n	x±Sx	VC,%	n	x±Sx	VC,%	n	x±Sx	VC,%	n	x±Sx	VC,%
FCR kg/kg	12	3.60±0.08	7.00	12	3.64±0.07	6.05	12	3.70±0.52	6.44	12	3.79±0.44	7.10

Table 4

Serum biochemical parameters in male Japanese quails after oral treatment with doxycycline for four consecutive days at a dose of 10 mg.kg^{-1} bw administered with or without silymarin extract. Level of significance: ** $p < 0.01$; *** $p < 0.001$; NS - Non Significant

		Triglycerides, TG (mg/dL)	ASAT (U/L)	ALAT (U/L)
Controls	x±Sx	13.95±0.76	454.33±4.69	44.00±2.18
	VC, %	19.57	21.98	32.43
Doxycycline	x±Sx	16.59±0.62	314.83±2.81	46.50±2.93
	VC, %	14.70	15.83	42.57
Doxy+silymarin 1%	x±Sx	19.42±0.39	313.50±2.88	39.50±5.10
	VC, %	8.51	16.26	80.07
Level of significance	P	1:2**; 1:3***; 2:3***	1:2,3***	NS

al. (2009) examined the herb-drug interaction of silymarin on the pharmacokinetics of trazodone in rats and found that high doses of silymarin (1.0 $g.kg^{-1}$) significantly decreased trazodone's area under the concentration curve (AUC).

In the present study we found that the values of FCR and body weight were not significantly changed in the groups treated with doxycycline and groups administered with silymarin extract compared to control group. Similarly to our results, Shiavone et al. (2007) found in their research that silymarin in broiler diets did not affect broiler growth whereas Blevins et al. (2010) and Mojahedtalab et al. (2013) pointed out that the supplementation with silymarin reduced feed consumption and conversion, but did not affect weight gain. Kalantar et al. (2014) reported that 0.5% of *Silybum marianum* extract supplemented to broiler diets did improve broiler performances. However, the results about the growth performance obtained were contradictory.

It is well known that silymarin extract have been used for protecting liver against diseases and intoxication. A number of 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 (Makki et al., 2014; Karvanmoghadam, 2014; Tahir et al., 2017). Furthermore, silymarin has very good radioprotector properties (Suchy et al., 2008). In our study three serum biochemical parameters were examined (triglycerides, ASAT and ALAT) as to see is there any influence of silymarin extract and a therapeutic dose of doxycycline on the liver function. Our results showed statistically significant increase in the values of triglycerides in the treated with doxycycline and silymarin groups in comparison to controls. 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 indicates an increase in metabolism, a reduction of hepatic storage of lipids or increased lipid mobilization. We found that the values of the serum enzymes ASAT and ALAT were decreased after treatment with doxycycline and silymarin supplementation in comparison to controls, statistically significant in ASAT values. Similarly to our results, Tahir et al. (2017) found lower values of ASAT and ALAT after 30 days supplementation of silymarin in Japanese quails. Suchy 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 ASAT and ALAT activity in blood plasma of the experimental groups of broiler chickens supplemented with *Silybum marianum* seed cakes was lower compared to the control groups. The treatment with chlortetracycline lead to slightly increased levels of ASAT and ALAT, which is similar to our results (Suchy et al., 2008).). In another study Vijayakumar et al. (2004) evaluated the silymarin's hepatoprotective effect on oxytetracycline induced hepatic disorder in dogs and found similar results to those reported by Suchy et al. (2008). Knowing that ASAT activity was found to be the most sensitive indicator of liver damage (Lumeij, 1997), we can conclude that the liver function was not damaged in our experimental conditions.

Conclusion

In conclusion, the simultaneous administration of silymarin extract and doxycycline in Japanese quails provoked a tendency of slightly decrease in serum concentrations of doxycycline which might be an evidence of herb-drug interaction. The obtained results of the current study might be a basis for further investigations evaluating more precisely the mechanisms of this interaction. According to our experimental conditions, no adjustment of dosage regimens of doxycycline was necessary when it is combined with silymarin extract. In the present study we did not find a positive impact of silymarin supplementation on growth performance of quails.

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