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Influence of the addition of milk thistle extract (*Silybum marianum* Gaertn) in compound feeds for fattening pigs on the biochemical indexes of blood and gain

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Abstract

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A scientific experiment for assessing the effect of added milk thistle extract *(Silybum marianum* Gaertn) on some biochemical parameters in the blood of fattening pigs with average live weight 39.813 kg – 39.827 kg was carried out. The animals are divided into two groups, each with fifteen pigs. The feeding of the experimental groups was done with feed with an equalized component composition, to the ration of the second group (experimental) a milk thistle extract was added in a dose of 10 g/day per animal. The additive in the combined feed of pigs during the first fattening subperiod has a positive effect on the health of the animals, expressed by changes in some basic biochemical parameters of the blood, good weight development and feed utilization.

The inclusion of 10 g/day per animal of milk thistle extract in the compound feed of fattening pigs significantly ($p \leq 0.05$) increased the average daily gain.

Keywords: milk thistle extract; biochemical blood indicators; pigs

Introduction

The European Union's 2006 ban on antibiotics in feeds as growth promoters created a trend to supplement pig feeds with biologically active substances that can have a beneficial effect on their health and production performance, while increasing nutritional value and palatability of the pork. Phytobiotics or organic chemical compounds from herbs, spices, fruits and vegetables are increasingly used as feed additives. The positive effects of herbs and spices are due to the high content of biologically active substances such as alkaloids, glycosides, flavonoids, essential oils, terpenes, organic acids and vitamins, which has been proven in a number of experiments (Nenova & Enchev, 2022; Pirgozliev et al., 2021a; Pirgozliev et al., 2021b; Pirgozliev et al., 2022). They strengthen the immune system and show anti-stress, antibacterial, antiviral, anti-inflammatory and antifungal effects (Liu et al., 2013). Due to its specific chemical composition and hepatoprotective effect, milk thistle is increasingly used as a feed additive for farm animals raised under intensive conditions (Liu et al., 2013; Grela et al., 2020). The healthy nature of this plant is due to the high content of biologically active compounds, such as taxifolin, quercetin, kaempferol, apigenin and eriodictyol. The main (1.5–3% dry matter) biologically active component of milk thistle is silymarin. It is an active complex of flavonolignans composed of silibinin (60%), isosilibinin (5%), silydianin (20%) and silicristin (10%). The silymarin complex shows a number of positive functions for the body: it traps free radicals, inhibits the formation of prostaglandins and inflammatory leukotrienes, regulates glutathione absorption and cell membrane permeability, which protects the liver from toxins entering the hepatocytes (Fanoudi et al., 2020). It promotes rRNA synthesis (a ribozyme that carries out protein synthesis in ribosomes) and inhibits the transformation of hepatic stellate cells into myofibroblasts, thereby regenerating the liver and protecting it from cirrhosis and fibrosis (Sridar et al., 2004). Silymarin reduces the expression of cytokine TGF-beta, normalizes blood glucose levels in hyperglycemic patients, supports the treatment of stress-induced gastric ulcers, and exhibits hypocholesterolemic and antiatherogenic effects (Simanek et al., 2011; Surai, 2015).

It has antihemorrhagic and antithrombotic properties, strengthens the walls of blood vessels and stimulates the secretion of bile and gastric juices, prevents the formation of gallstones and removes heavy metals from the body. Nutritional value is crucial for human dietetics and nutrition and is reflected not only in the ratio of the main chemical components, but also in the lipid profile and the content of essential and trace elements (Kropiwiec-Domańska et al., 2022). Research in recent years has also revealed the galactagogue effects of silymarin in animals and humans (Jiang et al., 2020).

Many experiments have been conducted to determine the effect of silymarin on pregnant sows. Jiang et al. (2020) found that silymarin supplementation during pregnancy and lactation could increase circulating concentrations of PRL (Prolactin), reduce oxidative stress, increase feed intake and improve protein metabolism, thereby significantly increasing milk yield of sows and subsequently improve the growth of their offspring. Other authors summarized an increasing dose-dependent effect on higher feed intake, reduced body weight loss, higher lactation and higher piglet weight at weaning (Zhang et al., 2021). Farmer et al. (2014) found that in female pigs, silymarin could increase prolactin concentrations and protect against oxidative stress, but the increase in prolactin was not sufficient to have a beneficial effect on mammary gland development in late pregnancy. In an experiment, the effectiveness of a multi-component herbal mixture used as a substitute for an antibiotic-premix in the feeding of pigs was evaluated. The herbal mixture consists of: *Melissa officinalis, Menthapiperita, Urtica dioica, Thymus vulgaris, Agropyron repens, Allium, Capsicum annum, Origanum maiorana, Coriandrum sativum, Taraxacum vulgare, Silybum marianum*. The results showed that pigs fed the herbal mixture supplement achieved the best average body weight gain (825 g per day) throughout the fattening period. This was 3.6% higher than the control group and 6.1% higher than the group receiving the antibiotic. The effectiveness of the herbal supplement is more pronounced during the first fattening period than during the second. Cooked meat from Longissimus dorsi of pigs receiving the herbal supplement was more tender and juicy compared to others with the other groups. The obtained results confirm the possibility of using the herbal mixture as an antibiotic substitute in pig feed (Hanczakowska et al., 2002).

The aim of the current study was to determine the effect of milk thistle extract (*Silybum marianum* Gaertn*)* on fattening pigs, while observing their health status, daily gain and biochemical parameters.

Material and Methods

At the Agricultural Institute – Shumen, a scientific experiment was conducted with a total of 30 pigs of the Danube White breed, divided into 2 groups of 15 each (experimental and control). The experiment started at an average live weight of 39.82 kg and ended after 30 days at an average live weight of 67.35 kg. Groups were equalized by number, live weight, sex, and origin. The food ration of the animals of the experimental group was supplemented with 10 g of milk thistle (*Silybum marianum*) extract.

The component composition of the feed and the content of energy and nutrients are presented in Table 1. Combined feeds for both groups are equal in terms of energy, protein, amino acids, calcium and phosphorus content. 10 g of milk thistle extract was added to the ration of the animals of the experimental group.

Pigs were housed and fed individually, ad libitum. They got water ad libitum with nipple drinkers. On day 0 and day 30

Table 1. Compound feed for fattening pigs

Components	%
Maize	23.88
Barley	5.00
Wheat	45.00
Soy meal	24.50
Premix	0.25
Synthetic lysine, 98%	0.07
Dicalcium phosphate	1.10
Salt	0.20
Total	100.00
OE, kcal	3122
OE, MJ	13.06
Crude protein, g	18.00
Crude fat, g	1.97
Crude fibers, g	3.38
Treonine, g	0.67
Tryptophan, g	0.24
Lyzine, g	0.95
Methionine + cystine, g	0.64
Ca, g	0.60
P, g	0.50

of the experiment, animals were weighed and blood samples were taken for biochemical parameters. The blood samples were taken from each pig from the orbital venous sinus using a closed system method. All samples were collected in plastic blood collection tubes (Vacuette). Serum biochemistry samples were allowed to clot at room temperature for 2–3 h before centrifugation (2000 × g for 15 min). Serum was collected and stored at −20°C for subsequent biochemical analysis. Analytical procedures were performed with a Selectra Pro XL automated biochemical analyzer (ELITech Group, Puteaux, France) in accordance with the manufacturer's instructions.

Standard methods of variance statistics (Microsoft Excel, 2010) were used when processing the results.

Results and Discussion

From the analysis of the blood samples, it was found that the addition of milk thistle extract to the ration of pigs during the first fattening sub-period has a positive effect on the biochemical parameters of the blood. The changes occurring within 30 days in the experimental group indicate the hepatoprotective effect of the herb, which is consistent with the findings of Abenavoli et al. (2018). A decrease in ALT values was observed in the animals from the experimental group ($p \leq 0.01$). Pares et al. (1998) proved that the consumption of 120 mg of silymarin twice a day for two months significantly reduced aspartate transaminase (AST) and alanine transaminase (ALT) in the blood serum of liver patients. According to a number of authors, its elevated values correlate very strictly with morphological changes of the liver. After the intake of milk thistle extract, the indicator's value significantly decreased, which indicates an optimization of the liver function, confirmed by the increased values of Glucose, which is in correlation with the studies of Kropiwiec-Domańska et al. (2022).

They found that milk thistle extract improved the biochemical profile of the liver and had a beneficial effect on fat and glucose metabolism, thereby reducing liver fat content. The liver and kidneys have the greatest glyconeogenetic capacity. Lactate, following the Corey cycle, passes from the muscles to the liver. Under the action of lactatoxidase, it is converted into pyruvate and forms α ketoacids with glycogen amino acids. They are transformed to oxaloacetate, which in the reverse pathway of the glycolytic chain is converted into glucose or glycogen. With a protected function of the liver, as it is in the experimental group, a rise in glucose synthesis was observed within the physiological norm. The study of total protein and its protein fractions is of great importance for the level of exchange processes and for the functional state of some organs and systems. Albumin is synthesized in the liver, there was a tendency to increase its value on day 30 in the experimental group (24.913-30.013; $p \leq 0.01$), which is in accordance with the stimulation of protein synthesis by regeneration of liver cells. Urea is a nitrogen product, and its content in the blood depends on the body's protein supply. A decrease in its value was observed, which indicates that the urea synthesizing function of the liver of animals from both groups is normal. Creatinine values show a tendency to increase within the physiological norm in the animals of the experimental group, in contrast to its values in the control group, where we observe a decrease. In the rat experiments of Ahmed et al. (2016) and Karimi et al. (2005), a decrease was found in Creatinine values. A positive trend towards a reduced level of serum Cholesterol and HDL-cholesterol was observed in the experimental group, which correlates with the hypocholesterolemic effect of the herb proven in the studies of Metwally et al. (2009) with rats. Skottová & Krecman (1998) demonstrated in their experiments that silymarin was associated with a reduction in hepatic cholesterol and this function was mainly due to fat-mediated enhanced bioavailability and/or by inhibition of dietary cholesterol resorption, which is consistent with Khazaei et al. (2022) who observed a similar result in Japanese quail. Silymarin is lipophilic and binds tightly to plasma membrane junctions, thereby increasing plasma membrane strength and preventing membrane rupture and disintegration (Basiglio et al., 2009). Clinical studies have shown that silymarin can be used as a cholesterol-lowering agent in hypercholesterolemic patients (Nassuato et al., 1991; Hoseini et al., 2004). It also has inhibitory properties against inappropriate blood fats (Schönfeld et al., 1997). By reducing cholesterol synthesis in the liver and lowering blood cholesterol by inhibiting its absorption in the gastrointestinal tract, silymarin may affect metabolism and blood fat concentration (Skottová et al., 2004).

The reported changes in the biochemical indicators of the blood tests in the control group are – lowered values of AST, urea and ALT, which can be interpreted as decreased liver function.

When comparing the changes in the biochemical indicators of the experimental and control groups, the optimized function of the liver is clearly visible in the animals receiving the milk thistle extract, which is correlated with the results of the increase reported for the period. (Tables 2 and 3).

Data on the weight development of the pigs are reflected in Figure 1. Statistically significant differences in growth were found between the control group and the group receiving the milk thistle extract. At the end of the experiment, it was found that the growth in the groups with the addition of silymarin in the feed exceeded the control. Pigs from the experimental group had a higher average daily gain by 15.94%, compared to the control group, and the differences were significant ($p \leq 0.05$).

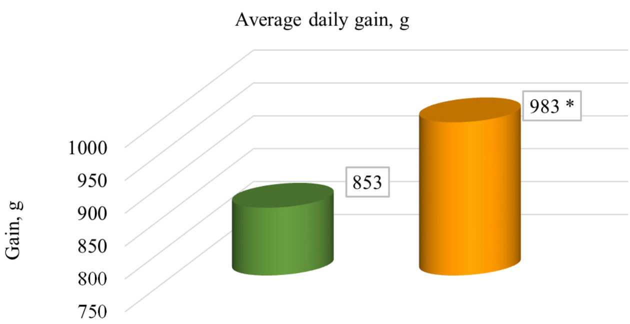
Table 2. Biochemical indicators – control group

Traits \ Group	I – day 0			I – day 30		
	$\bar{x}$	$S\bar{x}$	C	$\bar{x}$	$S\bar{x}$	C
AST U/L	37.807*	5.080	52.04	25.173*	2.076	31.94
LDL-Fr mmol/l	1.009	0.062	23.80	1.008	0.098	37.53
ALB g/l	23.013	1.119	18.83	26.173	1.501	22.21
UREA mmol/l	7.427***	0.445	23.20	5.220***	0.353	26.22
TRIG mmol/l	0.532	0.044	31.92	0.609	0.042	27.00
LDL-C mmol/l	1.320	0.064	18.70	1.401	0.102	28.19
ALT U/L	43.460*	4.531	40.38	30.747*	2.349	29.59
TPROT mmol/l	54.813	3.121	22.05	57.093	3.338	22.64
GLUC mmol/l	3.207	0.374	45.17	2.493	0.265	41.11
CREA mmol/l	76.533	2.171	10.99	74.067	3.836	20.06
CHOL mmol/l	2.463	0.108	16.95	2.300	0.142	23.87
HDL-C mmol/l	1.211	0.049	15.77	1.016	0.054	20.43

Table 3. Biochemical indicators – trial group

Traits \ Group	II – day 0			II – day 30		
	$\bar{x}$	$S\bar{x}$	C	$\bar{x}$	$S\bar{x}$	C
AST U/L	36.247	3.811	40.72	32.747	5.302	62.71
LDL-Fr mmol/l	1.157	0.076	25.28	1.129	0.120	41.21
ALB g/l	24.913**	1.004	15.60	30.013**	1.251	16.14
UREA mmol/l	6.760*	0.298	17.09	5.613*	0.304	21.00
TRIG mmol/l	0.547	0.050	35.52	0.690	0.050	27.89
LDL-C mmol/l	1.463	0.084	22.34	1.565	0.136	33.58
ALT U/L	45.260**	2.297	19.66	34.900**	2.247	24.94
TPROT mmol/l	58.993	1.916	12.58	61.840	2.350	14.72
GLUC mmol/l	2.345*	0.311	51.40	3.127*	0.213	26.44
CREA mmol/l	74.467**	2.582	13.43	83.667**	1.838	8.51
CHOL mmol/l	2.739	0.120	16.97	2.565	0.167	25.22
HDL-C mmol/l	1.335**	0.050	14.61	1.123**	0.048	16.63

Significance of differences – * – $p \leq 0.05$;** – $p \leq 0.01$;*** – $p \leq 0.001$



live weight	Group I - Control	Group II+SmG
at the beginning, kg	39,813	39,827
at the end, kg	65,400	69,307

Fig. 1. Average daily gain
* $p \leq 0.05$, SmG – *Silybum marianum* Gaertn

Conclusions

The addition of 10 g/g/den of milk thistle extract to the compound feed of "Danube White" breed fattening pigs has a beneficial effect on the health of the animals, expressed by some basic blood parameters and increases the reliability ($p \leq 0.05$) average daily gain of 39.813–39.827 kg to 65.400–69.307 kg.

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References

Abenavoli, L., Izzo, A. A., Milić, N., Cicala, C., Santini, A. & Capasso, R. (2018). Milk thistle (*Silybum marianum*): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. *Phytother Res.*, *32*(11), 2202-2213.

Ahmed, O., Mahmoud, A., Zid, S. & Saber, N. (2016). Silymarin and hydroethanolic extracts of *Silybum marianum* leaves and fruits attenuate Diethylnitrosamine/Phenobarbital-Induced Nephrotoxicity via their antioxidant and anti-inflammatory actions. *American Journal of Biochemistry*, *6*, 21-29.

Basiglio, C. L., Pozzi, E. J. S., Mottino, A. D. & Roma, M. G. (2009). Differential effects of silymarin and its active component silibinin on plasma membrane stability and hepatocellular lysis. *Chemico-Biological Interactions*, *179*(2–3), 297–303.

Fanoudi, S., Sadat, A. M., Karimi, G. & Hosseinzadeh, H. (2020). Milk thistle (*Silybum marianum*) as an antidote or a protective agent against natural or chemical toxicities: A review. *Drug Chem. Toxicol*., *43*, 240–254.

Farmer, C., Lapointe, J. & Palin, M. F. (2014). Effects of the plant extract silymarin on prolactin concentrations, mammary gland development, and oxidative stress in gestating gilts. *J. Anim. Sci.*, *92*(7), 2922-2930.

Grela, E. R., Swiątkiewicz, M., Florek, M. & Wojtaszewska, I. (2020). Impact of milk thistle (*Silybum marianum* L.) seeds in fattener diets on pig performance and carcass traits and fatty acid profile and cholesterol of meat, backfat and liver. *Livest. Sci.*, *239*, 10418.

Hanczakowska, E., Urbanczyk, J. & Świątkiewicz**, M.** (2002). The effect of addition of Probiodor on nutritive value of grower mixture in pig feeding. *Krmiva, 44*(1), 3-6. Retrieved from https://hrcak.srce.hr/139773**.**

Hoseini, H. F., Zaree, A. B., Babaei Zarch, A. & Heshmat, R. (2004). The effect of herbal medicine *Silybum marianum* (L.) Gaertn. seed extract on galactose induced cataract formation in rat. *Journal of Medicinal Plants*, *4*(12), 58–62.

Jiang, X., Lin, S., Lin, Y., Fang, Z., Xu, S., Feng, B., Zhuo, Y., Li, J., Che, L., Jiang, X. & Wu, D. J. (2020). Effects of silymarin supplementation during transition and lactation on reproductive performance, milk composition and haematological parameters in sows. *Anim. Physiol. Anim. Nutr.*, *104*(6), 1896-1903.

Karimi, G., Ramezani, M. & Tahoonian, Z. (2005). Cisplatin nephrotoxicity and protection by milk thistle extract in rats. *Evid. Based Complement Alternat. Med.*, *2*(3), 383-386.

Khazaei, R., Seidavi, A. & Bouyeh, M. (2022). A review on the mechanisms of the effect of silymarin in milk thistle (*Silybum marianum*) on some laboratory animals. *Vet. Med. Sci., 8*, 289-301.

Kropiwiec-Domańska, K., Babicz, M., Kędzierska-Matysek, M., Szyndler-Nędza, M., Skrzypczak, E. & Woliński, B. (2022). Effect of milk thistle (*Silybum marianum*) supplementation on pork offal quality. *Animals*, *12*(12), 1526.

Liu, Y., Song, M., Che, T. M., Almeida, J. A., Lee, J. J., Bravo, D., Maddox, C. W. & Pettigrew, J. E. (2013). Dietary plant extracts alleviate diarrhea and alter immune responses of weaned pigs experimentally infected with a pathogenic *Escherichia coli. J. Anim. Sci.*, *91*(11), 5294-5306.

Metwally, M. A. A., El-Gellal, A. M. & El-Sawaisi, S. M. (2009). Effects of silymarin on lipid metabolism in rats. *World Appl. Sci. J.*, *6,* 1634-1637.

Nassuato, G., Iemmolo, R. M., Strazzabosco, M., Lirussi, F., Deana, R., Francesconi, M.A., Muraca, M., Passera, D., Fragasso, A., Orlando, R., Csomos, G. & Okolicsányi, L. (1991). Effect of Silibinin on biliary lipid composition experimental and clinical study. *Journal of Hepatology*, *12*(3), 290–295.

Nenova, R. & Enchev, S. (2022). Influence of the inclusion of stevia (*Stevia Rebaudiana* Bertoni) in the compound feed of lactating sows on their haematological parameters. *Journal of Mountain Agriculture on the Balkans*, *25*(1), 1-14.

Parés, A., Planas, R., Torres, M., Caballería, J., Viver, J.M., Acero, D., Panés, J., Rigau, J., Santos, J. & Rodés, J. (1998). Effects of silymarin in alcoholic patients with cirrhosis of the liver: results of a controlled, double-blind, randomized and multicenter trial. *Journal of Hepatology*, *28*(4), 615-621.

Pirgozliev, V., Kljak, K., Whiting, I. M., Paul Rose, S., Mansbridge, S., Enchev, S., Atanasov, A. & Stringhini, J. H. (2021a). Feeding dry stevia leaf (*Stevia rebaudiana*) or xylanase improves the hepatic antioxidative status of broiler chickens. *Research in Veterinary Science*, *136*, 227-229.

Pirgozliev, V., Whiting, I. M., Mansbridge, S., Enchev, S., Paul Rose, S., Kljak, K., Johnson, A. E., Drijfhout, F., Orczewska-Dudek, S. & Atanasov, A. G. (2021b). Effect of rearing temperature on physiological measures and antioxidant status of broiler chickens fed stevia (*Stevia rebaudiana* B.) leaf meal and exogenous xylanase. *Current Research in Biotechnology*, *3*, 173-181.

Pirgozliev, V., Whiting, I. M., Kljak, K., Mansbridge, S., Atanasov, A., Paul Rose, S. & Enchev, S. (2022). Stevia (*Stevia rebaudiana*) improves carotenoid content in eggs when fed to laying hens. *Foods*, *11*(10), 1418.

Schönfeld, J. V., Weisbrod, B. & Müller, M. K. (1997). Silibinin, a plant extract with antioxidant and membrane stabilizing properties, protects exocrine pancreas from cyclosporin a toxicity. *Cellular and Molecular Life Sciences CMLS*, *53*(11–12), 917–920.

Simanek, V., Skottova, N., Bartek, J., Psotova, J., Kosina, P., Balejowa, L. & Ultrichowa, J. (2011). Extract from *Silybum marianum* as a nutraceutical a double blind placebo controlled study in healthy young men. *Czech J. Food Sci*., *19*, 106–110.

Skottová, N. & Krecman, V. (1998). Silymarin as a potential hypocholesterolaemic drug. *Physiol Res., 47*(1), 1-7, PMID: 9708694.

Škottová, N., Kazdová, L., Oliyarnyk, O., Večeřa, R., Sobolová, L. & Ulrichová, J. (2004). Phenolics – rich extracts from *Silybum marianum* and *Prunella vulgaris* reduce a high-sucrose diet induced oxidative stress in hereditary hypertriglyceridemic rats. *Pharmacological Research*, *50*(2), 123–130.

Sridar, C., Goosen, T. C., Kent, U. M., Williams, J. A. & Hollenberg, P. F. (2004). Silybin inactivates cytochromes P450 and 2C9 and inhibits major hepatic glucuronosyltransferases. *Drug Metab. Dispos.*, *32*, 587–594.

Surai, P. F. (2015). Silymarin as a natural antioxidant: An overview of the current evidence and perspectives. *Antioxidants*, *4*, 204–247.

Zhang, Q., Ahn, J. M. & Kim, I. H. (2021). Micelle silymarin supplementation to sows' diet from day 109 of gestation to entire lactation period enhances reproductive performance and affects serum hormones and metabolites. *J. Anim. Sci. 1*, *99*(12), skab354.

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