Planta Medica

Use of Milk Thistle in Farm and Companion Animals: A Review

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Conflict of Interest: The authors declare that they have no conflict of interest.

Abstract:
Milk thistle, Silybum marianum, is a medicinal plant grown for its bioactive compounds with well-documented antioxidant and hepatoprotective properties. Milk thistle has a well-established pharmacological reputation for treatments of human liver disease, but it is also used in animals. This review summarizes the experimental evidence of milk thistle's effects on animals when administered as silymarin extract (feed additive) or a feed ingredient, if administered as seed or expeller/cake with the seed residue still containing the bioactive components. The use as a feed additive or feed ingredient is motivated by the complexity of silymarin registration as a veterinary drug. In farm animals the drug improves the animals' performance and product quality and oxidative stability, supports liver function during productive life-cycle, improves gut-health and morphology, and can reduce intestinal pathogens. In dogs and cats the treatment is focused on acute and chronic liver diseases including the detoxification processes and support of drug treatments including chemotherapy. In equine athletes milk seed cake showed positive effects and a faster return of cortisol to the resting values before exercise occurred. In aquaculture it confirms its usefulness in supporting animal health and performance. In certain studies it is not clear what has been administered, and the composition and doses are not always clearly reported. A few studies reported no effects, but none reported problems connected to milk thistle administration. However, the overall picture shows that the use of milk thistle results in improved or restored health parameters or better animal performance.

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Contributors' Statement: Conception and design of the work: D.E.A. Tedesco; D.E.A. Tedesco and A. Guerrini were both involved with the collection, analysis and interpretation of data, as well as drafting and revision of the text.

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Thieme

Title

Use of Milk Thistle in Farm and Companion Animals: A Review

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Abstract

Milk thistle, *Silybum marianum,* is a medicinal plant grown for its bioactive compounds with well-documented antioxidant and hepatoprotective properties. Milk thistle has a well-established pharmacological reputation for treatments of human liver disease, but it is also used in animals. This review summarizes the experimental evidence of milk thistle's effects on animals when administered as silymarin extract (feed additive) or a feed ingredient, if administered as seed or expeller/cake with the seed residue still containing the bioactive components. The use as a feed additive or feed ingredient is motivated by the complexity of silymarin registration as a veterinary drug. In farm animals the drug improves the animals' performance and product quality and oxidative stability, supports liver function during productive life-cycle, improves gut-health and morphology, and can reduce intestinal pathogens. In dogs and cats the treatment is focused on acute and chronic liver diseases including the detoxification processes and support of drug treatments including chemotherapy. In equine athletes milk seed cake showed positive effects and a faster return of cortisol to the resting values before exercise occurred. In aquaculture it confirms its usefulness in supporting animal health and performance. In certain studies it is not clear what has been administered, and the composition and doses are not always clearly reported. A few studies reported no effects, but none reported problems connected to milk thistle administration. However, the overall picture shows that the use of milk thistle results in improved or restored health parameters or better animal performance.

Keywords: *Silybum marianum,* Asteraceae, silymarin, nutraceuticals, hepatoprotector, animal health

Abbreviations:

ALP: alkaline phosphatase

ALT: alanine aminotransferase,

AST: aspartate aminotransferase

BCS: body condition score

BHBA: β-hydroxybutyrate

bid: twice a day (bis/day)

BUN: blood urea nitrogen

BW: body weight

BWG: body weight gain

CAT: catalase

CCNU: chloroethylcyclohexylnitrosourea

CK-MB: creatine kinase isoenzyme MB

cTnI: plasma cardiac troponin I

d: day

DFI: daily feed intake

DWG: daily weight gain

Em: Egg mass

FCR: feed conversion ratio

FI: feed intake

GGT: γ-glutamyl transferase

GLDH: glutamate dehydrogenase

GPx: glutathione peroxidase

GSH: glutathione

GSSG: oxidized glutathione

h: hours

Hb: hemoglobin

HDL: high density lipoprotein

HSL: hormone-sensitive lipase

i.m.: intramuscular

i.v.: intravenously

IGM-2: immunoglobulin M-2

LDH: lactate dehydrogenase

LPS: lipopolysaccharide

m: month

MCP-1: macrophage chemotactic protein-1

MDA: malondialdehyde

Mn-SOD: manganese-superoxide dismutase

NEFA: non-esterified fatty acids

NF-κB: nuclear factor-kappa B

nr: not reported

p.i.: post-infection

PGE2: prostaglandin E2

PON-1: paraoxonase

PRL: prolactin

PUFA: polyunsaturated fatty acids

RBC: red blood cell

ROS: reactive oxygen species

SAMe: S-adenosylmethionine

SIL: silymarin

SOD: superoxide dismutase

TAC: total antioxidant capacity

TBARS: thiobarbituric acid reactive substances
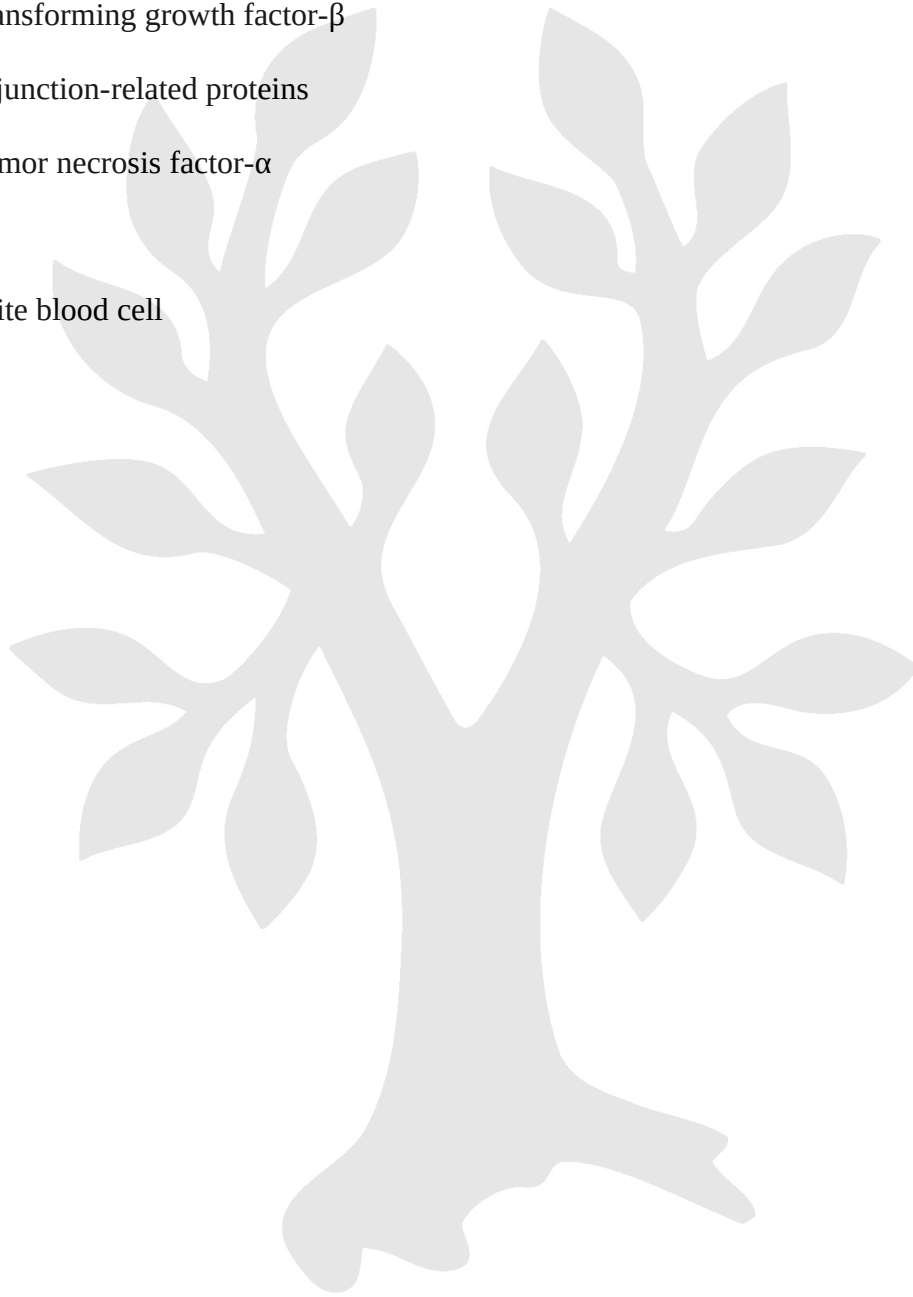
TG: triglycerides

TGF-β: transforming growth factor-β

TJs: tight junction-related proteins

TNF-α: tumor necrosis factor-α

w: week

WBC: white blood cell

Introduction

Milk thistle, *Silybum marianum* L. (Gaertn.), is a plant member of the Asteraceae family; it is widespread as a native or allochthonous species in different areas of the world. This interesting plant is very adaptable to many different growing conditions [1], and its crop species are cultivated prevalently as a medicinal plant in Eastern Europe as well as in Asia [2, 3]. The use of milk thistle is ancient, particularly as a supportive treatment of human liver disorders. It is also used as a traditional herbal medicinal product to relieve symptomatic digestive disorders including indigestion, to simulate the sensation of fullness, and to support liver function. The medicinal uses of milk thistle fruit were published in an official report issued by the European Medicines Agency (EMA) based on the published scientific literature [4]. The pharmacological potential of milk thistle is well documented by existing data that confirm the safety and tolerability of its herbal preparations in various settings related to hepatic disorders [4, 5]. The most important bioactive compound in milk thistle is SIL, a mixture of flavonolignans obtained from the processing of milk thistle fruit (botanically a fruit, like a seed), that has well-documented antioxidant and hepatoprotective properties [5-7]. The minimum content of SIL in mature milk thistle fruit is 1.5-2.0% [8] and often ranges between 1-8% of dry matter [1]. As mentioned above SIL contains different flavonolignans such as: silibinin (50-60%), isosilibinin (or isosilybin) (~5%), silycristin (~20%), silydianin (~10%), and other components such as taxifolin (~5%) [7]. Silybin or silibinin is the major and most active component in SIL. The greatest pharmacological activity is related to SIL itself. The mechanism of action by which SIL produces the clinical effects is attributed to its antioxidant activity, achieving the best results when the regenerative potential of the liver is still high thereby protecting intact liver cells or cells not yet irreversibly damaged [9]. The role of the drug in the treatment of liver diseases remains controversial. Part of this

uncertainty is due to the lack of data on its pharmacokinetics and optimal dosing regimens [10]. Because of the complexity of the absorption, metabolism, and disposition of various flavonoids, it is still unclear which form (i.e., the parent flavonoid or its metabolite/s) contributes to the overall effects in the body [10]. SIL is poorly water-soluble (solubility <50 µg/mL) [11] owing to its highly hydrophobic and non-ionizable structure, but it is slightly liposoluble in oil [12]. However, SIL has high permeability and is a class 2 drug based on Biopharmaceutics Classification System with low bioavailability after oral administration [13, 14]. This poor dissolution characteristic is the major obstacle in the preparation of effective oral formulations containing SIL. In the attempt to improve its bioavailability, many researchers in animals have reported studies concerning different types of preparations with silybin such as proliposome [15], self-microemulsifying, silybin-phospholipid complex [16], micelle [17], nanostructured [13], SAMe, or phosphatidylcholine [18, 19]. The pharmacokinetic parameters of SIL are referred to its principal active compound, silybin [7]. As evidenced in rats used as human models, after oral administration, silybin is rapidly absorbed from the stomach, but the absorption is rather low due to its limited solubility [20]. The limited solubility involves poor bioavailability, which is about 0.73% [21], caused by an extensive hepatic first-pass metabolism. This metabolic pathway is responsible for low oral bioavailabilities of different flavonoids including SIL [10]. The bioavailability of silybin was also studied in dogs [15, 19], cats [22], and horses [23] and confirms a low bioavailability even if SIL was administered complexed with lipid molecules [19, 21-23]. Silybin undergoes phase I and II metabolism, especially with conjugation reactions, producing different metabolites [20]. The reactions of phase I play a marginal role in the metabolism of flavonolignans in contrast to the reactions of phase II, which are very extensive, and hence, clearly dominant [20]. Most of the silybin goes through hepatobiliary excretion and

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enterohepatic circulation in the conjugated form and may be regulated by an active transport [20, 24]. Like conjugates, silybin is quickly excreted in the urine and faeces [20].

Other than in human treatment, milk thistle and its derivative products are successfully used to improve animal health and productivity. Some nutraceuticals are beneficial as they affect animal welfare, the maintenance of animal health, and healthy digestive systems, thus contributing to the reduction of chemical drug administration. Herbal treatments are targeted at a variety of different species with completely different backgrounds and preconditions. For example, in dogs and cats, the treatment patterns resemble those of humans while the treatment patterns for horses kept for sports purposes resemble those of human athletes. In farm animals, apart from the usual safety and efficacy studies, residues are considered to determine whether or not the consumption of food derived from these animals would be safe for humans. Nevertheless, as reported in human studies, the safety and tolerability of milk thistle is well documented [4]. There are stringent regulations on the use of nutraceuticals for farm and companion animals [25] in the European Union (EU). To collect information on the efficacy and safety of herbs and herbal products/botanicals, EFSA identified guidelines for their evaluation because these products can be included in animal nutrition of farm animals and carried over into the food chain [5]. Milk thistle can be administered as SIL extracts from the seed; in this case, SIL is an additive, and it is reported in Regulation (EC) No 1831/2003 [26] on additives for use in animal nutrition (sub-classification natural products botanically defined). If milk thistle seed is administered as a feed ingredient, it must comply with European Union (EU) Commission Regulation (EU) No 68/2013 [27], where it is reported in other seeds and fruits and products derived thereof. In the same case, milk thistle expeller, cake, or meal is considered a feed ingredient, as is the product remaining after the removal of oil/fat by pressing the seed. If milk thistle or SIL is administered with medical claims or due to the exertion of a pharmacological effect, its use must comply with the European legislation

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in the pharmaceutical sector for medicinal products for veterinary use according to Regulation (EU) 2019/6 [28]. The use as a feed additive or feed ingredient in animals is motivated by the complexity of SIL registration as a veterinary drug.

Search and Assessment Methodology

This review will analyze the scientific papers reporting evidence of the use of milk thistle on farm and companion animals. The literature research was performed by entering keywords in specific databases. In particular the search was conducted using Minerva, PubMed, Google Scholar, Scopus-Elsevier, Scifinder-n, and ResearchGate. The Minerva database is the access point to the bibliographic resources available from the University of Milan. The public portal of the European Medicines Agency (EMA) was used to obtain a herbal monograph on *Silybum marianum* based on available clinical evidence. No specific timeframe of publication years or language was considered for the research in the databases. The literature search was performed twice, in October 2021 and in April 2022. The search terms in databases consisted of the name of the animal species or zootechnical categories (i.e: swine including: *Sus scrofa domesticus*, sow, litters, pig, fattening pigs, etc). The terms "milk thistle," "*Silybum marianum*," "silymarin," and "silybin" were utilized to research the phytotherapeutic and phytoproduct description. In the first instance, the "use of silymarin in animal species" was inserted in the databases. The research was further refined for the ruminants category by inserting in the databases the key terms: "ruminant," "bovine," "*Bos Taurus*," "dairy cow," "steer," "*Capra hircus*," "goat," "*Ovis aries*," and "sheep." For poultry species and categories, the key terms were: "*Gallus gallus*," "poultry," "chicks," "broiler chicks," "broiler chickens," "hen layers," "laying hen," "*Cairina moschata*," "mulard," "duck," "*Coturnix coturnix*," "*Coturnix japonica*," and "quail." For the swine category the

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key terms were: "*Sus scrofa domesticus*," "swine," "sow," "litters," "piglets," "pig," and "fattening pigs." For the use of silymarin in aquaculture, the search key terms included "aquaculture" and "fish." For companion animals such as horses, dogs, and cats, the search key terms included, for horses: "equine," "*Equus ferus cavallus*," "horse," "sport horse," and "athletic horse;" and for dogs and cats: "canine," "dog," "feline," and "cat." The same key terms for each animal category were used and inserted in each previously cited database.

The selection of the studies was conducted following specific criteria. To be included publications had to provide at least one abstract written in English. The publications considered were *in vivo* studies including field trials on farms, experimental controlled trials, and *in vitro/ex vivo* studies on cells. In addition, studies on animals undergoing experiments with pathogens and studies on animals exposed to toxins different from mycotoxin were considered. The activity of milk thistle against mycotoxin-contaminated animals is not considered in this review because of the large number of papers on this topic that would require a topic-specific review. Publications investigating a mixture of different plant species in a combined preparation with *Silybum marianum* were excluded. In this review the studies on the effects of milk thistle (such as hepatoprotective, antidiarrheic, immunotropic, anti-inflammatory, antioxidant effects, and improved growth, improved feed conversion ratio, milk yield, etc.), or no effects, were considered.

Milk Thistle and its Derivative Products in Ruminants

The use of SIL extract from milk thistle was addressed regarding the problem of dairy cows around calving, late gestation, and the first three weeks of lactation, when the health and welfare of dairy cows are compromised by the subclinical fatty liver due to fat

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mobilization for energy unbalance. The primary challenge faced by cows in peripartum is a sudden and marked increase of nutrient requirements for colostrum and milk production at a time when dietary intake, and thus nutrient supply, lags far behind leading to a negative energy balance and to the mobilization of body fat in the form of NEFA. Extreme rates of lipid mobilization led to increased uptake of NEFA by liver and increased TG accumulation [29]. For dairy cows hepatic lipidosis is very common in the peripartum period from the influx of the large amounts of serum NEFA concentrations [30, 31]. The use of natural bioactive compounds as additives in farm animals instead of other chemical drugs was a natural consequence of the increased demand for safe products for human consumption [32]. In Table 1 the data relating to the use of milk thistle in ruminants species and categories are summarized. Among natural treatments, SIL is an acknowledged hepatoprotector used in humans to treat liver diseases and was tested to counteract the development of the fatty liver in dairy cows [33]. The human dosage was translated to dairy cows considering the animals' weight and the rumen with its large microorganisms interaction. The chemical structure of flavonolignans is not easily utilized by rumen microorganisms [34]. Krizova and co-workers [35] reported a flavonolignans rumen degradability ranging from 23.38 to 35.19%. In dairy cows [33], 10 g of SIL/d (silybin 49.1%, isosilybin 14.3%, silydianin 14.6%, silycristin 8.3%, taxifolin 4.3%) were administered from d 10 before expected calving to 15 d after calving. Treated animals showed a statistically significant peak of milk production 1 w before the control group with an average milk peak production of 41.6 ± 1.05 kg in treated animals, compared to 39.1 ± 1.44 kg for the control, leading to optimal milk production across the entire lactation (on 305 d, 9922.1 ± 215.7 kg for treated cows and 9597.8 ± 225.4 kg for control cows) even though SIL was administered for a short period of time. The use of SIL should not be considered without data on the safety and quality of productions intended for human consumption. An important element in ensuring the safe use of SIL in dairy cattle is

the monitoring of its residue in milk. After 10 g/d administration for more than a week, no silybin residue was detected (detection limit = 10 ppb). Plasma glucose, urea, TG, total cholesterol, BHBA and GGT were unaffected by treatment. After calving, the loss of BCS, the assessment of body fat, was lower for treated cows [36]. Histological examinations from liver biopsies showed variable quantities of fat in parenchymal cells in nearly all animals; in treated cows, hepatic fat was more conspicuous in parenchymal cells located near the central vein suggesting a more rapid fat mobilization from liver tissue to the circulatory system; and in control cows the hepatocytes with cytoplasmic vacuoles were widely located in the lobules and occupied their periportal zones [36]. In a similar experimental design, in periparturient dairy cows supplemented with 20 g/d of SIL (no details on the composition), the results above mentioned in milk production and BCS were confirmed [37]. The previous results were verified in field trials with dairy goats, and results were presented in several livestock scientific meetings. SIL administration to periparturient dairy goats (1 g/d: silybin 49.1%, isosilybin 14.3%, silydianin 14.6%, silycristin 8.3%, taxifolin 4.3%) from 7 d before the expected kidding date to 15 d postpartum significantly increased milk production on average from 0.61 to 0.92 kg/d in treated animals. In addition, for dairy goats, the milk quality parameters were not different among treated and untreated goats [38]. However, in dairy goats SIL promotes the accumulation of antioxidants in milk and prevents the oxidation of plasma protein. Milk levels of retinol were higher in treated goats, α-tocopherol was higher at d 7 post-partum in milk from treated goats, and plasma nitro-tyrosine, a measure of protein oxidation, was significantly lower in treated goats [39]. In addition histological investigations revealed an accumulation of fat in the liver of control goats, but no accumulation of fat was observed in SIL-treated goats [40]. The administration of SIL (1g/d: silybin 49.1%, isosilybin 14.3%, silydianin 14.6%, silycristin 8.3%, taxifolin 4.3%) was also assessed in middle-lactating dairy goats (third month of lactation). It was observed that SIL administration for 15

d, significantly increased milk production also at this stage of lactation with no effects on milk quality parameters. Furthermore, as observed in periparturient dairy cows and goats, milk yield remained higher after the end of the SIL administration and had no effect on milk quality parameters [41]. Lastly, it can be concluded that SIL administration in the peripartum period in dairy cows and goats may promote the metabolic adaptation of the early lactation period with better energy utilization and less BW loss; in dairy goats it prevented the hepatic fatty infiltration as well. Minimizing the problems associated with the transition period allowed dairy ruminants to express their milk potential yield. Only a few further studies reported the effect of SIL extract or milk thistle expeller in ruminants. In ketotic dairy cows fed milk thistle seed meal with the contents of 2.34% silybin and silydianin, a decrease in the sum of acetone + acetoacetic acid and β-hydroxybutyric acid in the blood, a drop in ketonuria degree, and higher milk production in treated animals [42] was observed. In steers the feeding of 0.50% diet with SIL in late fattening resulted in increased muscle mass and quality grade, in high BUN and creatinine, and in low ALT enzyme concentration, compared to the control animals [43]. In sheep, during a negative energy balance, the assumption of milk thistle expeller (200 g/d which provided 9 g of SIL) did not affect the blood biochemical parameters [44]. At a dose of 80 mg three times a day (composition not reported), SIL alleviated the pathogenesis of nephrotoxicity induced by gentamicin in sheep, reducing the renal damage makers protecting the kidney against antimicrobial toxic effects [45]. The use of SIL was tested *in vitro/ex vivo* to determine whether SIL has a protective effect on the primary hoof dermal cells of dairy cows. At a dose of 1 μg/mL (composition not reported), SIL may attenuate inflammatory responses in dermal hoof cells as reported in Table 1, opening up another opportunity to use SIL on bovine laminitis [46]. From the analyzed literature, the tested ranges reported for milk thistle seed cake were of 150-300 g/d, and for SIL, were of 1 g/d for sheep/goats and 10-20 g/d for dairy cow.

Milk Thistle and its Derivative Products in Poultry

In the literature most of the studies conducted on the use of milk thistle in poultry regard broilers and laying hens. Few studies were conducted on other poultry species such as quail (*Coturnix japonica*), ducks (Pekins, Mullard), or turkey broilers. It is known that the modern commercial broiler hybrids are genetically selected for rapid growth and are prone to oxidative stress (commonly heat stress), with the loss of redox homeostasis and production of ROS [47]. With the production of a high level of ROS, which exceeds the scavenging activity of the antioxidant defence systems, the oxidative stress results in the initiation and progression of hepatic damage as the liver is the main target tissue involved in responding to different sorts of oxidative stresses. The modern laying hen hybrids, characterized by high egg production, can be subject to oxidative stress and bone damage. The most significant damage is represented by hepatic injury as in the case of steatosis syndromes [48].

In poultry SIL appears to affect performance parameters though not systematically. In Table 2 data on the use of milk thistle in poultry species and categories are summarized. The administration of SIL in poultry modified, in some cases, the intestinal microflora and was positively correlated with the improvement of performance in the absorbing tract of the intestine. Recently, Jahanian and colleagues [49] noted increased DFI, average DWG, and FCR with SIL administration (500-1000 ppm feed, composition not reported) in *E. coli* challenge-broiler chicks. Dietary supplementation of SIL decreased the ileal *E. coli*, *Salmonella* spp., and *Klebsiella* spp. populations and increased the villus height and the villus height-to-crypt depth ratio in the *E. coli*-infected broiler chicks. These are possibly associated with the antibacterial property that protects the intestinal villi against the bacterial endotoxin enhancing the villi absorptive surface area [49]. Even in ducks a decrease of *E. coli* and an

increase of *Lactobacillus* spp. in cecal content with fed 0.6 g/kg SIL/diet (composition not reported) with an improvement of performance BW, BWG and FCR [50] was reported. In this study the authors concluded that SIL administration enhanced effects of beneficial bacteria in the large intestinal tract and improved the production of lactic acid, which may provide an energy source for intestinal epithelial cell growth, improving the nutrient absorption [50]. Shahsavan and co-workers [51] studied the effects of different levels of *S. marianum* oil extraction byproduct at a dose of 3%, 6%, 9% and 12% (total phenol content 206.18 µg/g and total flavonoid 571.62 µg/g) in the diet of broiler chickens. Dietary inclusion of 12% of milk thistle meal byproduct into the diet increased FI without differences in FCR and BWG among the treatment groups. The cecal population of *Lactobacillus* spp., Coliform, *E. coli*, total aerobes and Lactobacilli/*E. coli* ratio was not influenced by the integrations while the length of the duodenum, jejunum and cecum was increased in broilers fed at the levels of 9%, and 12% supplementation, presumably due to the high level of crude fiber (30%) in *S. marianum* oil extraction byproduct [51]. On the contrary Hashemi-Jabali and colleagues [52], testing incremental levels of milk thistle meal on laying hens, 30 and 60 g/kg of milk thistle meal (total phenolic component 470.64 mg gallic acid equivalent/g), led to a significant decline in ileal *E. coli* count. On jejunal morphology feeding 15 and 30 g/kg led to an enhancement in the villus height. However, at a dose of 60 g/kg, it exhibited undesirable effects on both villus height-to-crypt depth ratio and goblet cell numbers as a result of a mild stimulus mucosal of milk thistle bioactive substances. On performance, at a dose of 30 g/kg, the authors reported a decrease in FI, best FCR, and egg production (Em). Feeding incremental levels of milk thistle meal led to a remarkable decrease in serum cholesterol, TG, and MDA concentrations while there was a significant increase in blood high-density lipoprotein content [52]. On laying hens the effects on intestinal morphology derived from the administration of SIL were also observed by Faryadi and colleagues [53]. The feed

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supplementation with a different type-form of SIL (nano-SIL, powder-SIL, and lecithinized-SIL, composition not reported) affected the jejunal morphology, especially with the administration of nano-SIL and lecithinized SIL, made more bioavailable, with an increase in the villus height, villus width and villus to crypt ratio. The decreased intestinal pH values were also observed to be independent of SIL form and level. The crude protein and ether extract digestibility increased, regardless of the type-form of SIL. Additionally, possibly mediated by the effects of the drug on the gut development and/or retention of dietary nutrients, increased egg production (Em) and egg weight with no BW and FI variations were observed [53]. In Leghorn laying hens fed with a fish or sunflower oil supplemented diet, 30 g/kg of milk thistle meal (composition not reported) improved eggshell quality (strength, thickness and Haugh unit) with a reduction in egg yolk cholesterol. The milk thistle meal supplementation reduced the AST and ALT activities and serum MDA concentration. With respect to the liver, both hepatic relative weight and lipid percentages decreased, but the number of Kupffer cells increased. It seems that milk thistle meal has remarkable suppressive effects on hepatic MDA content with increasing highly PUFA dietary inclusion and the consequent lipid peroxidation in the liver [54]. The effects of milk thistle meal (taxifolin 580 mg/kg, silychristin 3638 mg/kg, silydianin 2520 mg/kg, silybin B 6673 mg/kg, silybin A 1473 mg/kg, isosylibin 565 mg/kg) in laying hen egg production was also reported with 7% of inclusion in the diet [55]. The authors reported a significantly higher egg production, higher Em, improved egg quality parameters, and higher antioxidant activity in the blood plasma [55]. A similar result was reported in Japanese quail, where an increase in laid eggs when a dose of 10 g/kg of milk thistle seeds in high calorie diets was administered [56]. With the supplementation of SIL at a dose of 0.5-1% (composition not reported) in the male Japanese quail diet, there were no highlighted differences on the main growth performance and some highlighted differences on hematological and biochemical parameters [57]. In a

study conducted on broiler chickens, where a basal diet was supplemented with 40-80 ppm of SIL (silychristin + silydianin 28.21%, silybin isomers 45.47%, isosylibin isomers 21.7%, and taxifolin 4.62%), the decrease of FI reported was ascribed to the reduction in palatability of the diet with SIL. As a consequence of the reduction in FI, carcass and thigh weight were negatively affected and could be responsible for the reduced content of lipid deposition in breast and thigh muscles. However, an increased muscle resistance to oxidative stress was observed with the reduction of TBARS [58]. Šťastník and co-workers [59], considering the meat quality, found that broilers fed with milk thistle seed cakes at a dose of 15% (flavonolignans 3.73%) had significantly improved breast tenderness and fibreness and flavour and colour parameters. However, 5 and 15% supplementation of milk thistle seed cakes caused a decrease in the average weight of chickens [59]. By comparison, when ground seeds of milk thistle were administered to broilers at percentages of 2 and 3% for the whole rearing period, the highest BW at the lowest FCR, a decreased crude fat in leg muscles and a positive effect on the meat flavour characteristics were reported [60]. However, Rashidi and colleagues [61] reported that the inclusion of *S. marianum* seeds (composition not reported) at a dose of 1, 1.5 and 2% in a broiler diet did not affect the carcass characteristics. They observed an increased FI with 1 and 1.5% of inclusion and BWG with 1.5% of inclusion. In turkey broilers SIL supplementation (0.5 and 1 kg/tonne, composition not reported) resulted in a higher final BW [62]. On the contrary, Suchý and colleagues [63] found that the addition of 0.2 and 1% milk thistle seed cakes (SIL 2.95%) in a broiler diet had no effect on live weight or FCR. Nevertheless, they observed a decrease in blood cholesterol and a decrease in the AST and ALT activity during the trial. Another study showed that the addition of 3% of milk thistle (no details reported) reduced blood uric acid, creatinine, cholesterol, and bilirubin [64]. Other blood parameters changed when milk thistle extract (SIL) was administered at a dose of 0.1, 0.5, 1.0, 1.5, and 2.0 mg/kg-BW in a broiler diet, increasing the number of RBC,

Hb, and WBC fractions, also increasing the γ-globulins as evidence of stimulation processes of hematopoiesis and of the immune system of SIL [65].

Recently, the effects of milk thistle were evaluated to reduce stress due to an increase in environmental temperatures. Starting with *in ovo*, followed by a dietary feeding of 100 mg/kg of milk thistle water extract during the growing phase of broiler chickens exposed to high temperatures (4 °C above optimum), milk thistle water extract led to increased FI, DWG, and final BW with the lowest FCR. An increased weight of bursa, thymus, spleen, and antibody titer against infectious bursal disease, and an increased H/L ratio were noted. The increased immunity response under elevated temperature was not improved starting with *in ovo* feeding of the extract [66]. Similar results were obtained when Gimmizah cockerels were fed with the fine grind aereal part of the milk thistle plant (1 g/SIL/kg diet), showing an improved bursa and liver weight, BW, and BWG, during summer season [67]. Again, in broiler chickens stressed by high temperature (38 °C), milk thistle supplementation at a dose of 10 g/kg feed contrasted the temperature effects, improving the BWG, FI, and FCR compared to control animals [68]. Similarly, Ahmad and co-workers [69] found that 15 g/kg of milk thistle seed supplementation significantly lowered the negative effects of natural summer stressed broilers on performance and oxidative stress. Regarding oxidative stress, Baradan and colleagues [70], in a study to investigate the hepatoprotective effects of SIL on CCl4-induced oxidative stress in broilers, indicated that SIL (100 mg/kg-BW, taxifolin 6.44%, silychristin 24.51%, silydianin 7.60%, silybin A 24.81%, silybin B 26.72%, isosilybin A 4.21%) has the potential to mitigate the deleterious effects of CCl4. These findings are supported by the fact that the protective activity of SIL against CCl4-mediated lipid peroxidation was demonstrated by the lower serum content of MDA as a lipid peroxidation marker. The study also confirmed that SIL reduced serum activity of ALP and AST hepatic enzymes. The ameliorating effects of SIL on CCl4 were also reported by Kamali and Mostafaei [71], finding a reduction of

ALT, AST, SGOT, and SGPT. In quail subjected to oxidative stress induced by CCl4, SIL administered at a dose of 1 mL/kg-BW (total phenol 2.60 mg/g, flavonoids 1.96 mg/g) reduced glucose, TG, and total cholesterol alleviating the adverse effects of CCl4 [72]. Evaluations of the effects of the administration of SIL were also carried out in ducklings exposed to oxidative stress induced by cumene hydroperoxide. In ducklings the supplementation with 100 or 200 mg/kg of SIL in the diet managed to limit the effects of oxidative stress. In intestinal mucosa, decreased MDA, increased SOD, GSH-Px activity, increased villus height of jejunum, and the ratio of villus height to crypt depth in duodenum, jejunum, and ileum probably resulted from the stimulated protein and DNA synthesis [73]. In broiler chickens treated against coccidiosis with Lasalocid coccidiostat, fed 800 mg/kg of SIL (Silimvet), counteracts the negative effects on ALT and significantly decreased the coccidiostat (Lasalocid) residues in liver and breast muscles thus reducing the risk from Lasalocid contamination of the broiler edible tissues [74]. From the analyzed literature, the tested ranges reported for the use of milk thistle seed cake in poultry were of 1.5-120 g/kg feed and for SIL, were of 0.1-2 g/kg feed.

Milk Thistle and its Derivative Products in Rabbits

There is a large body of literature on the husbandry and nutrition of rabbits used in commercial meat production systems and laboratory settings, with more limited information directly gained on pet rabbits [75]. Indeed, if they are socialized properly with humans while young, they are very friendly [76]. However, they are important farm animals; in fact, they are valued for their dietetic type of meat with its high-quality proteins and low fat and cholesterol content, suitable for human nutrition [77]. Digestive disorders account for 70% of rabbit diseases [78]. The exploitation of the positive effects derived from the SIL use against

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intestinal and hepatic disorders, oxidative stress and performance were tested in this species

as well. In Table 3, the data relating to the use of milk thistle in rabbits are summarized.

Kosina and co-workers [77] explored the effects of Silyfeed Basic, a commercial supplement

based on *S. marianum* (flavonolignans 4%, taxifolin 24.75 µg/g, silychristin 129.29 µg/g,

silydianin 19.43 µg/g, silybin A 68.56 µg/g, silybin B 119.52 µg/g, isosilybin A 31.33 µg/g,

isosilybin B 18.7 µg/g) at a dose of 0.2 and 1% in the diet for 42 d to evaluate the effects on

performance and oxidative status. No effects were noted on oxidative markers or other serum

biochemical parameters with the exception of increased serum cholesterol, total proteins, and

globulins at a dose of 1% of inclusion. Also, on oxidative markers, Attia and colleagues [79]

denoted in rabbit sperm and serum that SIL as milk thistle seed (total polyphenols equivalent

to gallic acid 392.1 mg/100 g) at a dose of 5-10 g/kg diet of rabbit bucks did not affect the

levels of antioxidant capacity, MDA, glucose, total lipids, total cholesterol, LDL, VLDL,

HDL, or TG. However, a reduction of FI and improvement of the blood serum and seminal

plasma testosterone in the treated group were observed. Indeed, ALT and AST hepatic

enzyme levels were reduced. No effects on the oxidative state of rabbit meat were noted by

Cullere and colleagues [80]. The supplementation with 5-10 g/kg of SIL powder

(composition not reported) for 11 w did not affect carcass traits and did not change the colour

or oxidative status of *Longissimus thoracis et Lumborum* muscle. Instead, it increased the pH

of this muscle, modifying the sensory traits with higher herbaceous odour [80]. The effect of

different milk thistle technology was evaluated in broiler rabbits [81]. Milk thistle seed

mechanically processed, fermented, and dried was compared with non-fermented milk thistle.

A positive effect on performance, DFI, total feed consumption, slaughter live weight, and

carcass weight were noted in the fermented supplement group compared to the non-fermented

milk thistle group [81]. For coccidiosis, an important parasitic disease in rabbits as in poultry,

SIL was tested at a dose of 100 mg/kg-BW (SIL 140 mg/capsule) in *Eimeria stiedae-*

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challenge rabbits [82]. From the treatment, no effects were noted on liver protection by damage of *E. stiedae*, performance, mortality rate, or biochemical parameters after 41 d p.i. Other studies focused on the protective effects of SIL after the application of drugs (anticancer, hypertension treatment or anticoccidial) with side effects causing hepatotoxicity. Jahan and colleagues [83] tested the effects of SIL in rabbits experimentally poisoned with isoniazid at a dose of 50 mg/kg once daily orally. At the same dose and pathway, SIL (composition not reported) was administered. They evidenced that the SIL-treated rabbits group increased the BW. In the isoniazid-treated group, the addition of SIL was able to arrest the decrease in the weight of rabbits even during the toxicosis status. However, no important changes in biochemical parameters were observed. From the analyzed literature, the tested ranges reported for the use of milk thistle seed cake in rabbits were of 5-10 g/kg feed and for the use of SIL were of 50-100 mg/kg-BW.

Milk Thistle and its Derivative Products in Swine

The potential effects of SIL were also tested in swine categories. Table 4 summarizes the data related to its use in swine and its production categories. Grela and colleagues [84] tested the effectiveness of the supplementation with 3 and 6% of milk thistle seeds (silybin A/B 26.4 g, isosilybin 5.3 g, silychristin 10.6 g, silydianin 0.1 g) for 100 d in fattening pigs to assess their performance traits. The addition of 3% milk thistle seed improved the DWG by about 2%, and 6% addition improved the gains by 3.8% during the growing period. However, for both doses administered, no significant effect on carcass meat content was evidenced. There was one exception; the backfat layer was thinner with a lowering-cholesterol effect, in particular in *Lumborum* muscle, backfat, and liver. An improvement of BW in piglets was noted also by Jiang and co-workers [85]. They reported that piglets from sows treated with 40

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g/d of SIL (silybin 10.32%, silydianin + silychristin 15.64%, isosilybin 6.91%) in the diet from d 108 of gestation (late pregnancy) to weaning evidenced a DWG and average weaning weight higher compared to the control group. This improvement of performance was explained by the higher colostrum yield and increased milk protein content from treated sows. In treated sows the serum concentration of PRL was increased on d 7 of lactation, and estradiol tended to increase. The authors reported that the increased production of PRL and estradiol appeared to be responsible for enhanced milk secretion in SIL-treated sows. The studies on the effects of SIL on the production of PRL hormone were conducted with conflicting results. The impacts of supplementing the diet for gestating gilts with 4 g/bid of SIL (silybin 49.4%, isosilybin 15.2%, silydianin + silychristin 35.4%) were studied from 90 to 110 d of gestation by Farmer and colleagues [86]. The authors showed that SIL tended to increase circulating concentrations of PRL at d 94 of gestation. However, the increase in PRL was not enough to have beneficial effects on mammary gland development in late gestation [86]. This effect remains partially unexplained. However, the SIL treatment caused a slightly upregulated effect on the *PPARGC1a* gene, a major gene implicated in mitochondrial biogenesis associated with mammary development and rate of milk synthesis. Even by increasing the dose administered (1 or 8 g/d of SIL extract with 11.4 and 17.3% of silybin A and B respectively) is administered to lactating sows throughout 20 d of lactation, no effects were noted on circulating concentrations of PRL and urea, oxidative status, performance, milk composition or piglet growth [87]. Similar results were evidenced by Loisel and colleagues [88]. When providing 1, 2 or 4 g/d of SIL (silybin 49.4%, isosilybin 15.2%, silydianin + silychristin 35.4%) for 8 d post-weaned cycling-sows, PRL concentrations did not increase, just as when supplying 12 g/d during the last 8 d of gestation, did not lead to hyperprolactinemia. The authors attributed these effects to a low intestinal absorption of SIL, as well as to a low dose administered, and a short duration treatment. Zhang and co-workers

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[16] administered varying doses of SIL coated by chitosan (SIL-micelle composed of 10.8% silybin, 16.3% silydianin, and 7% silychristin) at 0.05, 0.1, and 0.2% diet in periparturient sows, from the 109th prenatal d to the 21st postnatal d, and reported an improvement of the performance and biochemical parameters. In particular, FI, milk yields, serum hormones, and litter growth were evaluated. Proportionally, the AST enzyme decreased at the increase of SIL coated in the diet, supporting the hepatoprotective effect of SIL. The trial evidenced that, with the increase of the dose of coated SIL, litter weight and litter weight gain improved in w 1 and 2. The increase in average daily milk yields with uniform increases in fat content in SIL-treated sows was not associated with the BW loss. The same doses of SIL-micelle (composition not reported) were tested in fattening pigs [89]. The authors reported an improvement in FCR, BWG, and protein digestibility with the reduction of hydrogen sulfide and ammonia gas emission associated with an increase in fecal *Lactobacillus* spp. count at a dose of 0.2% diet. From the analyzed literature, the range of dose of milk thistle seed administered in swine was of 30-60 g/kg feed, and for only SIL was of 1-40 g/d.

Milk Thistle and its Derivative products in Aquaculture

As for other animal species, such as fish, there has been an attempt to reduce chemical drugs. In particular, in aquaculture, most research has been focused on the role of plant extracts in stimulating the immune system in fish, thus affording advantageous aqua feed nutraceutical additives. In Table 5, the data relating to the use of milk thistle in fish species are summarized. Banaee and co-workers [90] considered the inclusion of 100, 200, 400, and 800 mg/kg diet of SIL extracts (composition not reported) on juvenile rainbow trout to investigate the clinical effects and possible side effects of SIL on biochemical blood parameters. They observed a significant increase in protein levels in liver tissue of fish fed

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with SIL, an increase in plasma total protein and globulin concentrations, and higher total antioxidant levels in hepatocytes of treated fish with 400 mg/kg of SIL inclusion. Furthermore, the cholesterol levels in the plasma of treated fish were significantly lower. They concluded that 400 mg/kg had no side effects whereas 800 mg/kg induced a significant increase in AST activity, suggesting cellular damage. However, the authors do not clearly state the reasons for this evidenced hepatotoxicity. In another study on rainbow trout [91], to evaluate the immune response of animals, SIL (composition not reported) was administered into diets (0.1, 0.4, and 0.8 g/kg feed) of juvenile rainbow trout for 30 d. The administration of SIL for at least 15 to 30 d may increase the number of erythrocytes and leukocytes as well as haematocrit and Hb values. Hematological studies recorded an increase of RBC, hematocrit and Hb following the administration of SIL-supplemented feed. In other words, SIL may affect the function of haematopoietic organs such as spleen and head kidney, organs which play an important role in blood cell formation. A significant increase in plasma lysozyme activity of fish fed with enriched diets containing 0.1 and 0.4 g of SIL may indicate an improvement of defence mechanisms against bacterial agents. The enhancement of complement activity in plasma of fish fed with 0.4 g of SIL may indicate an improvement in the capabilities of the fish immune system [91]. As reported by Banaee and colleagues [90], a significant increase in the total plasma protein levels, particularly an increase of globulins in SIL-treated fish, may indicate an enhancement of their immune system. The authors concluded that the incorporation of SIL as an immunostimulant into fish feed might lead to enhanced health and immune parameters. Another circumstance in which milk thistle properties were considered involved the problem due to the polluted environment. SIL could be beneficial for alleviating deleterious impacts on fish growth in a contaminated environment. The efficiency of milk thistle in African catfish (*Clarias gariepinus*) growth in high fluoride water pollution was evaluated [92]. The authors administered a dose of 10 g/kg

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feed of dried milk thistle plant (seeds, leaves and stems included, composition not reported) to catfish exposed to fluoride. The study showed, in fluoride exposed fish, lower weight gain and a lower growth rate, a higher hepatosomatic index, and leukopenic and anaemic condition. By contrast the milk thistle addition significantly restored the normal hepatic architecture and reduced the oxidative stress and lipid peroxidation. Milk thistle enhanced the fish survival and reduced mortality. Treated fish showed a significant decrease in the extent and number of the fluoride-induced renal lesions associated with a significant improvement in the erythrogram and leukogram components. Hassan and colleagues [93] tested several levels of inclusion of milk thistle seed (taxifolin 2.9 g/kg, silychristin 2.5 g/kg, silydianin 1 g/kg, silybin A 1.7 g/kg, silybin B 2.6 g/kg, isosilybin A/B 0.84 g/kg) from 0 to 10 g/kg feed (SIL content from 0.0 to 123 mg/kg diet) on *Oreochromis niloticus* (L.) (Nile tilapia) fingerlings and fish. They aimed to evaluate the effects on growth performance, feed utilization, blood parameters, antioxidant enzyme activities, and gene expression. The total serum protein concentration, as well as albumin and globulin protein fraction contents, were higher in fish fed either 7.5 or 10 g of milk thistle (SIL: 92.25 and 123 mg/kg feed). The lowest level of AST and ALT enzymes; the highest total antioxidant enzyme activity of SOD and CAT; the highest up-regulated expression of SOD and CAT genes; and the highest transcripts accumulation of growth hormone in the pituitary were recorded in fish supplemented with 10 g/kg diet of milk thistle. However, the highest expression of IGM-2 was detected in the liver of fish supplemented with 7.5 g/kg of milk thistle. In their trial, milk thistle inclusion was beneficial for Nile tilapia regarding growth promotion, immune response modulation, and antioxidant enzyme capacity. Again, Owatari and co-workers [94] considered SIL feed additive as a hepatic protector and immunomodulator in Nile tilapia. After 55 d of SIL administration (16% of SIL), all fish were challenged with *Streptococcus agalactiae* to verify the effects on the immunological parameters and their protective effect

after the challenge. Before the challenge an increase of thrombocytes count was found in the supplemented fish. In the liver, dilation of the sinusoids was observed in non-treated fish only. In treated fish, however, the lesions were less severe. After the challenge, eosinophilic and lymphocytic infiltrate did occur in non-treated fish differently from supplemented fish, which did not show the alteration. The authors concluded that the administration of SIL as a dietary feed additive in the proportion of 0.1% developed an immunomodulatory framework with hepatoprotective effects in fish before and after the challenge with *S. agalactiae*. Another problem in aquaculture is the low availability of fish meal to be included in the diet. Substituting fish meal with increased content of plant protein in the diet can lead to reduced performance and disease resistance. Considering this statement Wang and co-workers [95] considered the inclusion of 100, 200, and 400 mg/kg of SIL (composition not reported) on growth performance, antioxidant capacity, and intestinal inflammation in juvenile turbot (*Scophthalmus maximus*) fed with a diet high in plant proteins. The results showed that the addition of 100 mg/kg of SIL significantly improved the growth performance. The antioxidant capacity in the liver was significantly implemented at a dose of 100 and 200 mg/kg, inducing the SOD and CAT activities, increasing the mRNA expression levels of SOD, GPx, and peroxiredoxin. Meanwhile, supplying 100 and 200 mg/kg enhanced the heights of villi and enterocytes. The supplementation reduced the mRNA expression of IL-8 and TNF-α, but it induced the expression of TGF-β in the intestine. These results indicated that adding 100 mg/kg of SIL enhanced the growth performance and health status of turbot fed with a high plant protein diet. Another constraint due to the fish diet can be the elevated lipid levels. In grass carp (*Ctenopharyngodon idellus*) the inclusion of 100 or 200 mg/kg of SIL (silibinin A/B 20.49 %, isosilibinin A/B 6.05 %, silydianin 3.38 %, silycristin 10.30%) was considered to counteract the high lipid content in the diet [96]. SIL inclusion enhanced growth performance, protein efficiency ratio, and feed utilization; reduced the hepatic lipid

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content, serum total cholesterol content, and the total bilirubin concentration; and notably reduced the elevated serum MDA content induced by the high level of lipids. Meanwhile, this group had higher interactions or up-regulated hepatic gene expression. Furthermore, in juvenile grass carp, SIL inclusion of 20, 40, 60, 80, and 100 mg/kg (SIL 95%) enhanced growth performance and promoted intestinal growth of fish. In particular, it reduced intestinal mucosal permeability and improved intestinal apparent morphology, acting on TJs [97]. It can be concluded that SIL improves health status in fish with hepatoprotective effects and reduces the increase in lipid peroxidation induced by high lipid intake. For fish, the analyzed literature reported a range of use of the milk thistle seed of 2.5-5 g/kg feed and 20-800 mg/kg feed of SIL.

Milk Thistle and its Derivative Products in Dogs and Cats

As with farm animals, the use of phytoextracts as nutraceuticals in the diets of pets, such as dogs and cats, has gained popularity in recent decades, in particular in veterinary medical practices [98]. Acute and chronic hepatobiliary diseases are quite commonly reported in both dogs and cats; however, in the veterinary literature, there is limited information on the hepatoprotective properties of SIL in pet animals [99]. Few *in vivo/ex vivo* studies are present in the literature resulting from the legislative difficulty of using dogs or cats for experimental purposes. In Table 6 the data relating to the use of milk thistle in companion animals, such as dogs and cats, are summarized. SIL has demonstrated, in pets, several beneficial actions useful in the treatment of hepatobiliary disease, including antioxidant, anti-inflammatory, and antifibrotic properties [100, 101], and with its supplementation, can serve as an effective therapeutical tool in pets with hepatopathies. Sgorlon and colleagues [102] noticed that the activity of ALT enzymes in 8 dogs clinically affected by liver damage, treated for 60 d with

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SIL (silybin 15%, 1.5 mg/kg-BW), was significantly reduced. At the reduction of the hepatic enzyme activity, a lower value of acute phase protein CuCp was also observed at a demonstration of the anti-inflammatory and antioxidant activity of silybin. The antioxidant activity of SIL was confirmed with the increase of PON-1 and by the significant SOD-2 up regulation. In another study, when SIL was administered at a dose of 28.3 mg/10 kg-BW of silybin (Hepaxan commercial product) according to the manufacturer's instruction to 15 dogs (20 ± 2 kg-BW) affected by liver disorders, the values of the enzymatic liver markers as AST, ALP, GLDH, and GGT decreased significantly. Furthermore, a slight increase in albumin and globulin concentrations was observed without influencing the values of LDH, α-amylase, or BUN and urea ratio [101]. However, when SIL was administered at a dose of 12.75 mg/kg-BW (Hepaxan, commercial products) in healthy beagles for 28 d, the levels of IL-4 and IL-10, mean corpuscular hemoglobin concentration, bilirubin, GGT, and TG increased, which confirms that this supplement improved liver metabolism. In other biochemical parameters, the authors observed a decrease of WBC, neutrophils, eosinophils, ALP, glucose, and α-amylase. With minor relevance, an alteration of the serum fatty acid profile (lower concentration of C20:5-n3 and C22:0, and higher concentration of C15:0 and C17:0) was observed [103]. In the same study, in a dog group with hepatopathies, Hepaxan reduced the hepatic miR-122 gene expression [103]. The hepatic damage caused by toxic agents was studied in 1990 by Paulova and colleagues [104], who tested the efficacy of SIL (100 mg/kg-BW/bid) for treating CCl4-induced liver disease in 16 beagles. SIL was administered 4 d before CCl4 (used as a toxic agent) and 4 d after. Both as preventive and post-intoxication treatment, it proved to be almost ineffective to protect the liver from damage. Some products such as Denamarin, a commercially available product containing a complex with stable salt of SAMe and silybin in a phosphatidylcholine complex (S-adenosylmethionine 64.04%, silybin A/B 12.49%), were efficacious in dogs treated with CCNU for chemotherapy in a

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randomized trial conducted by Skorupski and colleagues [105]. In 68% of dogs (17/50 dogs) the concurrent administration once per day of Denamarin was efficacious to reduce the effects of CCNU on both hepatocellular damage and biliary dysfunction. Furthermore, in a study conducted by Kocatürk and co-workers [106], in 5 healthy dogs treated daily with SAMe 20 mg + silybin 1 mg/kg-BW, when exposed to bacterial LPS to cause endotoxemia at a dose of 2 µg/kg-BW i.v., the increase of liver enzymes associated with the effects of LPS were inhibited at 1-24 h by the treatment with SAMe + silybin. In another study, Soltanian and colleagues [107] compared the therapeutic effects of SIL to hydrocortisone on clinical and hematological alterations and organ injury (liver and heart) when dogs were exposed to a low dose of LPS. They noticed a significant increase in RBCs, Hb, and HCT, and a significant decrease in AST, ALP, LDH, CK-MB, and cTnI in SIL-treated group [107]. The hepatic or renal parameters, altered in clinical cases of severe hepatic or renal damage, were reported to physiological values in case of emergency administration of SIL (composition not reported) at a dose of 20 mg/kg in dogs with kidney damage caused by the administration of gentamicin at a dose of 20 mg/kg/d [108]. The same effects were observed in cats at a dose of 30 mg/kg-BW of SIL in cases of liver damage caused by stanozolol [109], phenobarbital [110], tetracycline [111], and mebendazole [112]. Unlike dogs, cats are extremely sensitive to the toxic effects of some molecules because of their hepatic enzyme deficiency, in particular the glucuronyl transferases. However, this relative deficiency of the glucuronide conjugation pathway results in more drugs being conjugated to sulfates, but the sulfation pathway has a finite capacity in cats, which is also lower than in other species [113]. At a dose of 30 mg/kg-BW, SIL had the same effect as *N*-acetylcysteine in the treatment of liver damage from acetaminophen intoxication in cats. In SIL treated cats, the levels of ALT, AST, LDH, methemoglobin and bilirubin did not increase as they did in cats given acetaminophen alone [113]. At a higher dose than 70 mg/bid, SIL was also shown to be effective when

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administered together with amiodarone, contrasting amiodarone's antiarrhythmic actions and preventing sustained atrial flutter by reduction and/or elimination of the excitable gap in dogs, probably due to the antioxidant effect of SIL [114]. In a study conducted by Webb and co-workers [115], the administration of 10 mg/kg-BW/d orally of a silybin-phosphatidylcholine complex (silybin 31%) for 5 d in cats increased the GSH content and phagocytic function in cats challenged with *E. coli*.

Few studies were conducted *in vitro/ex vivo*. The effects of the combination of SAMe + silybin (298 ng∕mL) on primary canine hepatocytes exposed to the pro-inflammatory cytokine IL-1β studied by Au's research group [116] revealed that this combination was associated with reduced levels of PGE$_2$, IL-8, and MCP-1, a pro-inflammatory molecule induced by IL-1β administration, produced by macrophage associated with higher GSH levels with a resulting reduction of oxidative stress. In another study, the same authors tested the effects of SAMe (30 and 2000 ng/mL) with Samsyl (silybin: 298 ng/mL), noting a decrease of NF-κB factor in primary canine hepatocytes culture associated with a reduction of PGE$_2$, IL-8 and MCP-1 levels [117]. The study demonstrated that the combination of SAMe + silybin acts on two principal pathways involved in the defence of hepatocytes, specifically against oxidative stress and inflammation. The authors concluded that SAMe + silybin may ameliorate activation of the inflammatory and oxidative stress cascades initiated by the IL-1β, and an attenuation of NF-jB translocation from the cytoplasm to the nucleus, associated with a reduction in gene transcription of COX-2, chemokines and GSH. For dogs, the analyzed literature reported a range of SIL at a dose of 10 mg/kg-BW and of 10-140 mg/kg-BW, while in cats, a dose of 30 mg/kg-BW was reported.

Milk Thistle and its Derivative Products in Horses

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In general, horses are raised both for meat consumption and sporting purposes. In Table 7, the data relating to the use of milk thistle in horses are summarized. The oral bioavailability of silybin was tested in horses by the administration of silybinin complexed with phospholipids (32.7% silybin phospholipid) both by nasogastric tube and via feed [23]. From serum analyses, the bioavailability of oral administration of silibinin phospholipid was < 1%, confirming that the bioavailability was low in horses, as in other species, regardless of the use of silibinin complexed with phosphatidylcholine. Intensive physical exercise of equine athletes significantly affects physiological and biochemical processes. The consequences of intensive physical exercise are reflected in the condition and health of individuals, assessed objectively by blood biochemical changes. In this regard, in a study conducted by Dockalova and colleagues [118], SIL was administered in the form of milk thistle seed cakes (SIL 13.4 g/d) up to 400 g/d to equine athletes exposed to intensive physical exercise (regular combined driving training) for 56 d. The monitored blood biochemical parameters corresponded to the reference range of values except for slightly increased creatine-kinase and decrease of AST and ALP enzymes at 56 d and a dose of 400 g/d of integrated feed. However, immediately after physical exercise, blood parameters such as AST and NEFA decreased, and haematic phosphorus increased, suggesting that treated horses proved a better use or recovery of energy sources. During the entire period of the trial, cholesterol, HDL and LDL in treated horses, increased and cortisol levels decreased, despite the intensive exercise. SIL provided by administration of milk thistle seed cakes had a positive effect on horse health and energy metabolism, proven by lower NEFA values, with higher utilization of NEFA during exercise, associated with a faster return of cortisol to the resting values. In another study conducted by Dockalova and colleagues [119], mares were fed with different daily feed doses of milk thistle expeller (100, 200, 400 and 700 g/d) with a SIL content of 3.4, 6.8, 13.4 and 19.4 g/d respectively, to evaluate the digestibility of SIL,

monitored by HPLC-UV from faeces samples. The authors evidenced statistically significant differences on digestibility of flavonolignans, ALT, and creatinine.The most suitable daily dose seemed to be 400 g/d of milk thistle seed cakes, also in mares, as with equine athletes. Also, the apparent silybin digestibility increased with a gradual increase of milk thistle seed cakes in the feed dose, with silybin B, which had an average higher digestibility compared to silybin A [118]. Vigorous physical exercise or pathological status in horses can cause inflammation, such as laminitis [120], or secondary inflammation from bacterial infection such as sepsis [121], which is one of the most frequent causes of morbidity and mortality in horses. For the study of the effects of SIL on laminitis, *in vitro/ex vivo* model could be used. For this purpose, lamella of hooves are explanted and consisted of 6–8 intact epidermal lamella junctions and 2 mm of dermal connective tissue, cultured in well plates with a medium at 37 °C and 5% CO_2 [122]. Probably due to the antioxidant and anti-inflammatory effects of SIL in the laminitis model with bacterial infections, the endotoxins were neutralized, and LPS was reduced to induce lamellar separation [122]. Zoloblenko and co-workers [123] investigated the effects of silybin, taxifolin, and dehydro-silybin (composition not reported) on ROS production in vitro in equine neutrophils. The dehydro-silybin inhibited superoxide production and myelo-peroxidase release modulating the oxidative response involved in the pathogenesis of laminitis. Furthermore, the effect of silybin on LPS induced inflammatory responses in equine PBMC denoted that at doses of 10 µM and 50 µM in equine PBMCs, silybin was able to prevent the LPS induced increased levels of TNF-α, IL-1β, IL-6, and IL-8, further indicating that this pharmacological approach could be useful in treatment or prevention of several inflammatory conditions in horses [124]. In horses the literature reported the use of milk thistle seed cake in a range dose between of 100-700 g/d and of 13-42 mg/kg-BW of silibinin.

Conclusions

Of the 80 studies analyzed, only 30% of the studies reported the composition of milk thistle product tested, expressed as SIL and/or the flavonolignans content. A few reported no effects, but none reported problems connected to milk thistle administration. However, the quality of the studies is improving, and the overall view of the documents showed many positive results using milk thistle to improve health parameters, restore health, reducie disease risk, and improve animal performance. On animal performance, many parameters were enhanced by the use of milk thistle: FI, FCR, BWG, and BW. Furthermore, an enhancement of animal product yields and quality were reported in several trials. Animal studies do not clarify the exact mechanism of action of milk thistle. However, on different animal species, its administration showed an improvement of liver functionality with a reduction of liver enzymes activity (AST, ALT, GGT, etc). Many studies highlighted different effects on biochemical parameters such as the WBC, RBC, Hb, and H/L ratio. Another reported result was that nutrient utilization probably improved due to the improved gut morphology. The effect on gut microbiota was reported in several papers and showed that milk thistle can improve worthy bacteria (i.e., lactic acid bacteria) with a reduction of pathogenic bacterial populations. The up-regulation of gene expression of anti-inflammatory markers (TNF-α, interleukins) following milk thistle administration was also reported in some studies. The antioxidant effect was reported as a reduction of several parameters such as low MDA, SOD, and CAT levels, and higher GSH activity. Despite the reduced research studies, milk thistle is mostly sold in various forms of supplements for dogs and cats, often without details on bioactive components, especially as liver support, and for the detoxification process.

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Good results were reported when the animals received milk thistle cake/expeller because the bioactive compound remained in the seed after the oil was removed. Therefore, in the areas where milk thistle is cultivated and processed, the use of milk thistle expeller/cake as a feed ingredient is a valuable strategy. Due to the limits of the availability of milk thistle expeller/cake, the opportunity to improve the use of milk thistle in animals can be in the form of SIL added to the diet as feed additives. To ensure the evidenced effects derived from the administration of SIL and its efficacy and usefulness as an additive in animal feed, it is important that the SIL be marketed as a feed additive reporting, at least, the amount in the additive. That way, the expected effects would be guaranteed for the farmers and animal owners.

Conflict of Interest

The authors declare no conflict of interest.

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Table 1. Effects of the administration of milk thistle and its derivative products in ruminant species. The tested dose refers to diet inclusion if not diversely reported.

Animals Categories	Milk thistle type	Tested dose	SIL content	Time of treatment	Effects	References
Ruminants						
Cows						
Dairy cows	Milk thistle fruit expeller	150 g/d	SIL 4.10% Silychristin 10.45 g/kg Silydianin 1.51 g/kg Silybin A 9.24 g/kg Silybin B 15.1 g/kg Isosilybin A 3.48 g/kg Isosilybin B 1.11 g/kg Taxifolin 2.39 g/kg	29 to 32 w of lactation	Digestibility of silybin A and B was 40.0 and 45.5%, respectively. Rumen degradation of taxifolin was 59.11%, other flavonolignans ranged from 23.28 to 35.19%. Milk yield or composition was not affected by the treatment	[35]
Dairy cows (peripartum)	SIL (INDENA)	10 g/d	Silybin 49.1% Isosilybin 14.3% Silydianin 14.6% Silycristin 8.3% Taxifolin 4.3%	~25 d (10 d before expected calving) to 15 d after calving	Peak of milk production 1 w before; peak milk production 41.6 kg vs. 39.1 kg; less body condition loss; greater milk production throughout entire lactation (9922.1 ±215.7 vs. 9597.8 ±225.4 kg). No effects on blood and milk parameters	[33]
Dairy cows (peripartum)	SIL (INDENA)	10 g/d	Silybin 49.1% Isosilybin 14.3% Silydianin 14.6% Silycristin 8.3% Taxifolin 4.3%	~25 d (d 10 before expected calving) to 15 d after calving	Liver biopsies: in control cows, the hepatocytes with cytoplasmic vacuoles were widely located in the lobules; in treated cows, fat-rich hepatocytes were conspicuous near the central vein and may be related to a more rapid fat mobilization from liver tissue to the circulatory system. Clinical chemistry values were similar for both groups	[36]
Dairy cows (peripartum)	SIL	20 g/d	nr	3 w before expected calving to 3 w after calving	Increase in milk yield, decrease of milk protein; less body condition loss. SIL supplementation increases the ALT activities, not related to liver damage	[37]

Dairy cows	Milk thistle seeds	0.3 kg/d	Silybin+ Silydianin 2.34%	ketotic dairy cows 15 d	Decrease of acetone + acetoacetic and beta-hydroxybutyric acid in the blood. The ketonuria degree dropped remarkably. Higher milk production. The treated group maintained positive effects after treatment stopped	[42]
Steers						
Hanwoo steers	SIL	0.50%	nr	steers in late fattening from 25 to 32 m of age	Higher blood urea nitrogen and creatinine. Low serum ALT concentration. Non-differences on final weight. Improve the meat quality parameters	[43]
Goats						
Dairy goats (peripartum)	SIL (INDENA)	1 g/d	Silybin 49.1% Isosilybin 14.3% Silydianin 14.6% Silycristin 8.3% Taxifolin 4.3%	from 7 d before calving until 15 d after calving	Milk yield of treated animals was significantly higher with respect to control (increase 0.61-0.92 kg/d for each animal). No differences in milk quality parameters	[38]
Dairy goats (peripartum)	SIL (INDENA)	1 g/d	Silybin 49.1% Isosilybin 14.3% Silydianin 14.6% Silycristin 8.3% Taxifolin 4.3%	from 7 d before calving, until 15 d after calving	Plasma titres of retinol and α-tocopherol did not differ between the two groups. Higher level of nitro-tyrosine only in control goats. Higher levels of retinol and α-tocopherol in treated goats	[39]
Dairy goats (peripartum)	SIL (INDENA)	1 g/d	Silybin 49.1% Isosilybin 14.3% Silydianin 14.6% Silycristin 8.3% Taxifolin 4.3%	from 7 d before calving until 15 d after calving	Liver biopsies: variable sizes of lipid vacuoles were present in control dairy goats compared to treated goats, where no lipid vacuoles were present in hepatocytes	[40]
Dairy goats	SIL (INDENA)	10 mL/d	Silybin 49.1% Isosilybin 14.3% Silydianin 14.6%	third month of lactation 15 d	Increase in milk production.Milk yield was maintained higher in the treated group also after the end of the treatment. No effects on milk quality parameters derived by the treatment	[41]

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			Silycristin 8.3% Taxifolin 4.3%			
Sheep						
Sheep	Milk thistle seed cake	200 g/d	SIL 9 g	sheeps in negative energy balance 3 w	Higher level of GGT. No toxic effects. No effects on energy balance	[44]
Sheep	SIL	80 mg/animal i.m., for 3 times/d	nr	20 d	In gentamicin-induced renal toxicity, as measured by multiple functional, structural and enzymatic factors, SIL reduced the toxic effects.	[45]
***in vitro/ex vivo* primary hoof dermal cells of dairy cows**	SIL	0.1-20 μg/mL	nr	24-48 h	Decreased the secretions of IL-1β, and TNF-α, inhibited the phosphorylation of p65 NF-κB and p38 MAPK, and promoted the mRNA gene expressions of CYP3A4 and CYP1A1 in the LPS-induced dermal inflammatory model. Potential positive effects on bovine laminitis	[46]

Table 2. Effects of the administration of milk thistle and its derivative products in poultry species. The tested dose refers to diet inclusion if not diversely reported.

Animals Categories	Milk thistle type	Tested dose	SIL content	Time of treatment	Effects	References
Poultry						
Laying hen						
	Milk thistle meal	15-30-60 g/kg	Expressed as: Total Phenolic component 470.64 mg gallic acid equivalent/g	80 d	At 30 g/kg: best FCR, higher Em, reduction of ileal *E. coli* enumeration, an enhancement in the villus height-to-crypt depth ratio; decrease in serum cholesterol, TG and MDA concentrations; increase in blood high-density lipoprotein content and goblet cell numbers	[52]

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	Milk thistle seed cake	7%	Taxifolin 580 mg/kg Silychristin 3638 mg/kg Silydianin 2520 mg/kg Silybin B 6673 mg/kg Silybin A 1473 mg/kg Isosylibin 565 mg/kg	11 w	Higher number of eggs, more Em. Higher Haugh units, higher millimetre height of the egg and thinner eggshell. Higher blood antioxidant activity	[55]
	SIL Nano-SIL Powdered-SIL (PSM) Lecithinized-SIL (LSM)	100 mg/kg-BW 200 mg/kg-BW each type	nr	12 w	At 200 mg/kg of nano SIL or LSM improved the hen performance and egg quality: increased egg production, egg weight, Em and Haugh unit scores, crude protein, and ether extract digestibility; decreased FCR. The villus height, villus width, and villus to crypt ratio were improved. With nano-SIL there was a reduction in of the crypt depth	[53]
	Milk thistle cake	15-30 g/kg	nr	80 d	At 30 g/kg there was an improvement of eggshell strength, thickness, and Haugh unit. In hens fed with a diet with fish or sunflower oil there was a reduction of serum or egg cholesterol concentrations and blood or hepatic MDA content	[54]
Broiler						
	SIL	0.5-1%	nr	42 d	Average DFI, ADWG, and FCR were improved. The villi absorptive surface area was increased, such as jejunal villi height and villi height to crypt depth ratio. There was also a reduction in crypt depth. Decrease of *E.coli, Salmonella* spp. and *Klebsiella* spp. count, after challenge	[49]
	Milk thistle cake	3-6-9-12%	Expressed as: Total Phenol 206.18 µg/g Total Flavonoid 571.62 µg/g	42 d	d 8 to d 21: at 3%, 9% and 12% increase of FI, BWG. d 22 to d 42: FI increase and better FCR. At the levels of 6%, 9%, and 12% there was an improvement of the length of the duodenum, jejunum, and cecum. At d 16, a higher value is recorded for wing web thickness in birds that received 9% in the diet only at 24h following the phytohemagglutinin-P injection. The higher value is observed for cutaneous basophilic hypersensitivity response at 12 and 24 h post-injection on d 21 and d 35 fed by 9%	[51]

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Milk thistle seed	2-3%	nr	42 d	Increase of BW and decrease of FCR. No effect on the carcass composition. Reduction respectively the content of crude fat in leg muscles. Higher content of PUFA	[60]
Milk thistle seed	1-1.5-2%	nr	42 d	Highest FI in the starter and finisher period observed at a dose of 1 and 1.5% of inclusion. Highest BWG at a dose of 1.5% The highest relative weight of gizzard and intestine were in the 0.5 and 2 % of inclusion. The lowest cholesterol, TG, VLDL and the highest level of HDL at a dose of 1.5%	[61]
Milk thistle water extract	100 mg/kg	SIL (≥ 80%) Silybin isomers (≥ 30%)	42 d	Highest FI, daily WG, final BW and lowest FCR. At 28 and 42 d, increase of weight of bursa, thymus and spleen with an increase in antibody titer against infectious bursal disease. WBC count was higher with a lower H/L ratio. At d 28 but not at 42 d, there was an increase of Na^+ e K^+ levels	[66]
Milk thistle seed cake	5-15%	Flavonolignans 3.73%	37 d	Higher breast meat tenderness, optimal sensory quality traits, decrease of growth of chickens	[59]
Milk thistle extract	0.1-2%	nr	40 d	Increase of RBC, Hb, WBC and the concentrations of serum γ-globulins. Decrease of albumin level	[65]
Milk thistle	0.3-3%	nr	42 d	Effects on the levels of uric acid, blood creatinine and bilirubin	[64]
Milk thistle grind aerial parts	12.5 (0.5 g of SIL/kg-feed) 25 g (1 g of SIL/kg-feed)	188.5 mg of total polyphenols and 320.0 mg of antioxidant activity/100g	70 d	Respect to the control group, milk thistle plant addition improved FI, BW, and BWG (in particular with the addition of 25 g), followed by the group supplied with Vitamin E, and FCR. Improved weight of liver, spleen, bursa of Fabricius (in particular with the addition of 25 g of milk thistle plant.) Improved all blood biochemical constituents (AST, ALT and ALP) and significantly decreased lipid profile (total lipids, triglycerides and cholesterol). Diets supplied with different doses, significantly improved TAC,	[67]

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				GSH, MDA, H/L ratio, phagocytic activity and phagocytic index	
	Milk thistle	10 g/kg	nr	3 w	In broilers exposed to heat stress (38°C), the SIL treated group showed a higher FI and BWG with better FCR and an increase of digestibility. The WBC, RBC, and Hb were improved compared to the control group	[68]
	Milk thistle seed	5-10-15 g/kg	nr	42 d	In natural summer stressed broilers (26-37°C), in supplemented group with the 15 g/kg, FI, BWG, and FCR were improved. A lower level of serum MDA was detected	[69]
	SIL (Silimvet)	800 mg/kg	SIL 80%	49 d	In coccidiostats + SIL treated animals, Lasalocid residues decreased in liver and breast muscles. No change in biochemical parameters, except for ALT, that decrease in SIL + Lasalocid treated groups	[74]
	SIL (Sylimwet)	0.5-1 kg/tonne	nr	--------	In treated animals: higher final BW in broilers, turkey-hens, and turkey-cocks, and increase of hatchability in broiler breeders and the number of hatching eggs. The SIL treatment prevented an excessive adiposis in birds	[62]
	Milk thistle seed cake	0.2-1%	SIL 2.95%	52 d	No effects in BW and FCR. Lower cholesterol, lower ALT and AST values at d 22, and only ALT at d 52. Decrease of the content of lipids and increase of the content of glycogen in the liver of both experimental groups	[63]
	SIL Plusil (BIOTRADE)	40-80 ppm	Taxifolin 4.62% Silychristin+ Silydianin 28.21% Silybin isomers 45.47% Isosylibin isomers 21.7%	60 d	No effect on growth performances but slightly affected slaughtering yields negatively. No specific hepatoprotective effects. Reduction of the lipid content of both breast and thigh and increase the muscles resistance to oxidative stress	[58]
	SIL	60 ppm	nr	6 w	In CCl4-treated groups ALT, AST, GOT and SGPT enzymes are	[71]

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	Plusil (BIOTRADE)				reduced by SIL. The activity of cytochrome P450 is reduced by the treatment with SIL	
	SIL	100 mg/kg-BW	Taxifolin 6.44% Silychristin 24.51% Silydianin 7.60% Silybin A1 24.81% Silybin B1 26.72% Isosilybin A 4.21%	42 d	A remarkable down-regulation in the expression of CAT, Mn-SOD and GPx hepatic genes in CCl4-challenged birds. Liver damage was amended by SIL	[70]
Quail						
	Milk thistle seed	1%	nr	3 w	In animals fed with high-calorie basal diet: higher total egg production, best yolk colour, and lower MDA level in kidney, with the protection of kidney against free radical damage. Reduce GSH and GSH-Px levels activity in kidney	[56]
	Milk thistle extract powder	0.5-1%	nr	40 d	In male Japanese quail, no change in biochemical parameters, exception of a tendency of higher values of TG and lower values of AST and ALT	[57]
	SIL	1 mL/kg-BW	Total phenol 2.60 mg/g Flavonoids 1.96 mg/g	42 d	The interaction effect between SIL and CCl4 ameliorate the adverse effects of CCl4-induced oxidative stress: SIL did not affect productive parameters whereas increased of serum total protein and decrease glucose, TG and total cholesterol	[72]
Duck						
	SIL seeds extracts	0.6-0.9-1.4 g/kg	nr	70 d	At 0.6 g/kg diet improved growth performance (FCR, BW and BWG), antioxidant status (GSH and SOD), liver functions markers, immune responses, and there was a decrease of lipid fractions, increase of *Lactobacillus* spp. and decrease of *E. coli* of cecal contents	[50]
	SIL	100-200 mg/kg	SIL 80%	50 d	The toxic effect induced by cumene hydroperoxide was reduced in	[73]

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					intestinal mucosa: with SIL treatment, intestinal mucosa increase the activity of SOD and GSH-Px. In jejunum, increase mucosal DNA, RNA and protein and increase the villu's height and the ratio to cripth-depth	

Table 3. Effects of the administration of milk thistle and its derivative products in rabbits. The tested dose refers to diet inclusion if not diversely reported.

Animals Categories	Milk thistle type	Tested dose	SIL content	Time of treatment	Effects	References
Rabbits						
	Milk thistle Silyfeed Basic	0.2-1%	Flavonolignans 4% Taxifolin 24.75 µg/g Silychristin 129.29 µg/g Silydianin 19.43 µg/g Silybin A 68.56 µg/g Silybin B 119.52 µg/g Isosilybin A 31.33 µg/g Isosilybin B 18.7 µg/g	42 d	Data showed a mild effect on the growth performance of rabbits. Total protein, globulin and total cholesterol increased at a dose of 1%. No effects on oxidative stress markers	[77]
	SIL	5-10 g/kg	nr	42 d	The meat sensory characteristics (herbaceous odour) were reported, but not in other carcass traits; There were no effects on performance	[80]
	Milk thistle	0.5% fermented/dried 1% non-fermented	nr	43 d	Fermented: positive effects on performance, DFI, total feed consumption, slaughter live weight, and carcass weight	[81]
	SIL	100 mg/kg-BW	Legalon (140 mg capsule)	41 d	In SIL-treated/*Eimeria*-infected group: ALT levels decrease, hyperproteinemia, and decrease of albumin content. SIL did not have antiparasitic activity against *E. stiedae,* mitigated the adverse effect of coccidiosis infection	[82]

| | Milk thistle seed | 5-10 g/kg | Total Polyphenols (equivalent to gallic acid) 392.1 mg/100 g | 18 w | Improvement in the rabbit buck semen quality and fertility like sperm concentration, total sperm output, live sperm, total live sperm and total motile sperm. Decrease of FI and serum ALT and AST enzymes | [79] |
| | SIL | 50 mg/kg-BW | nr | 19 d | SIL did not affect ALT enzyme and bilirubin levels. On liver damage by isoniazid, SIL improved serum bilirubin and serum ALT concentration | [83] |

Table 4. **Effects of the administration of milk and its derivative products in swine categories. The tested dose refers to diet inclusion if not diversely reported.**

Animals Categories	Milk thistle type	Tested dose	SIL content	Time of treatment	Effects	References
Swine						
Pig	Milk thistle seeds	3-6%	Silychristin 10.6 g Silydianin 0.1 g Silybin A/B 6.4 g Isosilybin A/B 5.3 g	100 d	Improved DWG, FCR, and PUFA content in tissues, water-holding and antioxidant capacities. Increase of the hypocholesterolemic index in all tissues and reduced the thrombogenic and atherogenic indices	[84]
Pig	SIL micelle	0.05-0.1-0.2%	nr	10 w	Improved average daily gain and trend to increase the FCR at w 10. Increase in the digestibility of nitrogen at w 5 and 10. Linearly increased the faecal *Lactobacillus* spp. count at w 5 with the reduction of the hydrogen sulfide and ammonia gas emission at w 5 and 10. Linear reduction of the blood cholesterol level at w 10. At the end of the trial, supplemented pig had linearly improved meat colour. The inclusion of 0.2% was found to be the optimal level to enhance the growth performance, nutrient digestibility, faecal microbial, faecal gas emission, and meat quality of finishing pigs	[89]
Sow (peripartum) /piglets	SIL coated chitosan (micelle)	0.05-0.1-0.2%	Silybin 10.8% Silydianin 16.3% Silychristin 7%	d 109 of gestation to weaning	Increased litter weight and litter WG. Sows: increased FI, BW loss decreased, milk yields and fat content (d 14). Decreased AST and epinephrine concentration, and activity of GSSG. Higher activity of	[16]

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Animals Categories	Milk thistle type	Tested dose	SIL content	Time of treatment	Effects	References
					SOD and GSH-Px	
Sow (peripartum) /piglets	SIL	40 g/d	Silybin 10.32% Silydianin + Silychristin 15.64% Isosilybin 6.91%	d 108 of gestation to weaning	Piglets: higher daily gain and weaning weight. Sows: higher CAT (d 18 of lactation), GPx (d 7 of lactation) and PRL (d 7 of lactation). Lower concentration of TNF-α (d 7 of lactation) and IL-1β (18 d of lactation)	[85]
Sow	SIL (BIO-C)	1-2-4 g/d	Silybin 49.4%, Isosilybin 15.2% Silydianin plus Silychristin 35.4% (BIO-C provided by INDENA)	8 d starting 24 to 48 h after the onset of standing heat	No effects on circulating concentrations of PRL, progesterone, estradiol-17β or leptin in sows	[88]
Sow/piglets	SIL (BIO-C)	8 g/d	Silybin 49.4%, Isosilybin 15.2%, Silydianin plus Silychristin 35.4% (BIO-C provided by INDENA)	from 90 to 110 d of gestation	Tendency (p > 0.10) to increase of circulating PRL concentrations at d 94. No effects on oxidative status, performance and on their litter	[86]
Sow/piglets	SIL	1-8 g/d	Silybin A 11.4% Silybin B 17.3%	20 d of lactation	No effects on urea, PRL, oxidative status, and piglets growth	[87]

Table 5. **Effects of the administration of milk thistle and its derivative products in fish species. The tested dose refers to diet inclusion if not diversely reported.**

Animals Categories	Milk thistle type	Tested dose	SIL content	Time of treatment	Effects	References
Aquaculture						
***Oncorhynchus mykiss* (Trout)**	SIL	100-200-400-800 mg/kg feed	nr	28 d	No changes in performance parameters. At 400 mg/kg: reduction of plasma glucose and cholesterol levels. Increase of plasma total protein, globulin and albumin	[90]

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					levels. Stabilization of cellular membrane structure and regulate the levels of AST, ALT, ALP, CK, and LDH activity. At 800 mg/kg is noted a cytotoxic effect and modifications in blood biochemical parameters	
Oncorhynchus mykiss (Trout)	SIL	100-400-800 mg/kg	nr	30 d	At 100 mg/kg: increase of leukocyte and thrombocyte count, lysozyme activity, albumin and globulins level. At 400/800 mg/kg: increase of RBC, thrombocytes, Hb, hematocrit, protein and plasma protein, lysozyme activity (400 mg/kg), and globulins concentration (800 mg/kg)	[91]
Oreochromis niloticus (Nile tilapia)	Milk thistle seeds	2.5 g/kg (SIL: 30.75 mg/kg) 5 g/kg (SIL: 61.5 mg/kg) 7.5 g/kg (SIL: 92.25 mg/kg) 10 g/kg (SIL: 123 mg/kg)	Taxifolin 2.9 g/kg Silychristin 2.5 g/kg Silydianin 1 g/kg Silybin A 1.7 g/kg Silybin B 2.6 g/kg Isosilybin A/B 0.84 g/kg	70 d	Supplementation with 7.5 and 10 g/kg recorded the highest FBW, WG and best FCR. No difference in dry matter content of the whole fish body; increase of protein and ash content at 7.5 and 10 g/kg, lipid content, total antioxidant capacity of SOD and CAT activity. Decrease of hepatic enzyme activity. Improve GH hormone gene activation	[93]
Oreochromis niloticus (Nile tilapia)	SIL phosphatide	0.1% (1 kg/tonne)	Heptarine S , composed of 16% SIL phosphatide	54 d	No alteration in the feeding behaviour and growth and hematological parameters, but there was a higher total count of thrombocytes value in treated fish, neither before nor after challenge with *S. agalactiae.* No differences in morphometry of the intestine and pancreas acini structure (also after challenge). The eosinophilic and lymphocytic infiltrates are more severe in the control group	[94]
Ctenopharyngod on idellus (Grass carp)	SIL	20-40-60-80-100 mg/kg	SIL 95%	70 d	Improvement of growth performance and promoted intestinal growth; reduction of the intestinal mucosal permeability and improved intestinal apparent morphology; ameliorated intestinal partly related to the enhancement of barrier forming TJs and AJ-related proteins mRNA levels and the reduction of pore-forming TJs mRNA levels through suppressing RhoA/ROCK signalling pathway (RhoA/ROCK/MLCK/NMII) in the	[97]

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					intestine	
Ctenopharyngodon idellus (Grass carp)	SIL (78%)	100-200 mg/kg	Silibinin A/B 20.49% Isosilibinin A/B 6.05% Silydianin 3.38% Silycristin 10.30%	82 d	At 100 and 200 mg/kg: higher WG, SGR and lower FCR with lower hepatic lipids content. Increase of finally BW, WG, specific growth rate, protein efficiency ratio and protein productive value, and a decrease of FCR with the addition of high concentration of lipids. Decreasing tendency of serum HDLc contents with an increase of the dietary lipid levels. No effects on serum TG, NEFA, HDL, and glucose concentrations. Increase of mRNA of CPT1, HSL, HMGCR and CYP7A1 genes expression	[96]
Clarias gariepinus (African catfish)	*S.marianum* whole plant (seeds, leaves, stems) dried	10 mg/kg	nr	60 d	SIL improved the FBW, BW, DWG, and SGR, but reduced the hepatosomtic index. Increase of RBCs, WBCs, lymphocytes, heterophils, eosinophils, and monocytes count as well as Hb content and packed cell volume percentage serum levels of complement, nitric oxide, total proteins, albumin, and γ-globulin. A significant decline in the serum levels of hepatic enzymes (AST, ALT, ALP) urea and creatinine were also observed. Liver and kidney histopatholgy showed normal histological pictures, but basic alteration in all samples was hepatocyte swelling with lipid and/or no-lipid cytoplasmic vacuolation were identified	[92]
Scophthalmus maximus L. (Turbot)	SIL (~80% pure)	100-200-400 mg/kg	nr	9 w	At 100 mg/kg: improved the growth performance, but no effects on feed utilization. There was an enhancement of the heights of villi and enterocytes. The antioxidant capacity in the liver was improved also at 200 mg/kg, by not only inducing the activities of SOD and CAT but also increasing the mRNA expression of SOD, GPx and peroxiredoxin. Reduction of the mRNA expression of IL-8 and TNF-α but induced the expression of transforming TGF-β in the intestine	[95]

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Table 6. **Effects of the administration of milk thistle and its derivative products in dogs and cats. The tested dose refers to diet inclusion if not diversely reported.**

Animals Categories	Milk thistle type	Tested dose	SIL content	Time of treatment	Effects	References
Companion						
Dog (different breeds) with hepatic disorder	Silybin	28.3 mg of Silybin/10 kg-BW	Hepaxan	30 d	The values of the enzymatic liver markers as AST, ALP, GLDH and GGT, decreased significantly. A slight increase in albumin and globulin concentrations was observed without influencing the values of LDH, α-amylase, BUN and urea ratio	[101]
Healthy beagle and with hepatic disorder	Silybin	12.75 mg of Silybin/10 kg-BW	Hepaxan	28 d	Increase of the level of IL-4 and IL-10, mean corpuscular hemoglobin concentration, bilirubin, GGT and TG. Decrease of WBC, neutrophils, eosinophils, ALP, glucose and α-amylase and reduction of the miR-122 gene expression	[103]
Dog with hepatic disorder	Milk thistle	10 mg/kg-BW (1.5 mg/kg of Silybin)	Silybin 15%	60 d	Reduce the hepatic enzymes activity (ALT/GPT) and increase the PON-1. There was an up regulation of the SOD-2	[102]
Healthy dogs	Silybin	SAMe (20 mg) - Silybin (1 mg) kg-BW	Denamarin Phosphatidylcholine complex: S-adenosylmethionine 64.04% + Silybin A/B 12.49%	24 h	Serum blood urea nitrogen and creatinine increase at 1–24 h after LPS challenge. LPS-induced clinical and haematological changes (increases in liver enzymes) however attenuated by SAMe and silybin treatment	[106]
Healthy dogs	SIL bolus	SIL bolus 10 mg/kg i.v., in LPS-induced sepsis model	SIL (Sigma-Aldrich)	24 h	In canine induced-sepsis, SIL reduce the AST, ALP, LDH and plasma cardiac troponin I values, with an improvement of Hb	[107]

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					and HTC levels	
Healthy dogs	SIL	SIL 70 mg/bid + Amiodarone 600 mg/d	SIL Legalon	8 w	SIL potentiated amiodarone's antiarrhythmic actions and prevented sustained atrial flutter by reduction and/or elimination of the excitable gap reducing the free radical generate by amiodarone's toxicity	[114]
Healthy dogs	SIL	Gentamicin 20 mg/kg/d, SIL 20 mg/kg/d	SIL (Sigma-Aldrich)	9 d	Total serum antioxidants activity was greater, decrease of serum MDA concentrations, and a low serum creatinine in SIL treated group with gentamicin-induced nephrotoxicity	[108]
Dog (chemotherapy with CCNU)	S-adenosylmethionine 64.04% + Silybin A/B 12.49%	Manufacturer's instructions (once/d)	Denamarin: Phosphatidylcholine complex: S-adenosylmethionine 64.04%+ Silybin A/B 12.49%	6 m	Mitigate the effects of CCNU, and reduction of hepatic enzymes	[105]
in vitro/*ex vivo* **model dog hepatocytes**	Silybin and Silybin–phosphatidylcholine complex (SPC)	298 ng/mL	Silybin and SPC	24 h	IL-1ß, cytokine used as a pro-inflammatory stimulus. Pretreatment with SB and SPC significantly inhibited IL-1ß-induced production of pro-inflammatory markers-attenuated NF-$_k$B nuclear translocation	[116]
in vitro/*ex vivo* **model dog hepatocytes**	Silybin	S-adenosylmethionine (SAMe; 30 and 2000 ng/mL) and Samsyl (SB) : 298 ng/mL), with the combination of IL-1ß-induced inflammation and oxidative stress	SAMe and Samsyl (SB)	24 h	SAMe and SB combination inhibits both inflammation and oxidative stress: reduced cytokine-induced PGE2, IL-8 and MCP-1; inhibited NF-$_k$B nuclear translocation; increased of antioxidant enzyme-reduced GSH	[117]

Healthy cats	Silibinin-phosphatidylcholine complex (SPC)	10 mg/kg-BW/d	Silibinin 31%	5 d	Increase of the mean fluorescence intensity in feline lymphocytes and granulocytes, representing of GSH content; the percentage of phagocytic cells responding optimally significantly increased	[115]
Healthy cats	SIL	SIL 30 mg/kg-BW/d + Phenobarbital 16 mg/kg-BW orally	SIL (Sigma-Aldrich)	28 d	The results showed that SIL can protect liver tissue against oxidative stress in cats with phenobarbital intoxication, especially in the first 3 h post-exposure	[110]
Healthy cats	SIL	SIL 30 mg/kg-BW/d + Tetracycline 120 mg/kg-BW orally	SIL (Sigma-Aldrich)	28 d	The results showed that SIL can protect liver tissue against hepatotoxicity in cats with tetracycline severe overdose, particularly in the first 4 h post-exposure; levels of serum enzyme activities (ALT, AST, ALP, LDH) remained within the normal values	[111]
Healthy cats	SIL	SIL 30 mg/kg-BW/d + Mebendazole 200 mg/kg-BW orally	SIL (Sigma-Aldrich)	28 d	The results showed that SIL can protect liver tissue against oxidative stress in cats with mebendazole intoxication, particularly in the first 2 h post-exposure; levels of serum enzyme activities (ALT, AST, ALP, LDH) remained within the normal values	[112]
Healthy cats	SIL	SIL 30 mg/kg-BW + Acetaminophen single dose of 150 mg/kg-BW	SIL (Sigma-Aldrich)	24 h	SIL can protect liver tissue against oxidative stress in cats with acetaminophen intoxication. Levels of serum enzyme activities, methemoglobin, and total and direct bilirubin remained within the normal values	[113]

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Table 7. **Effects of the administration of milk thistle and its derivative products in horses. The tested dose refers to diet inclusion if not diversely reported.**

Animals Categories	Milk thistle type	Tested dose	SIL content	Time of treatment	Effects	References
Horses						
Horses	Silibinin phospholipid and pure Silibinin	0-6.5-13-26 mg/kg-BW/bid orally via feed via nasogastric tube, and i.v.	Silibinin phospholipid (INDENA) Silibinin (Sigma-Aldrich)	4 w	Silybinin had low bioavailability, and did not accumulate when administered bid for 7 d	[23]
Mares (not pregnant)	Milk thistle seed cakes	100 200 400 700	3.4 g/d 6.8 g/d 13.4 g/d 19.4 g/d	15 w	Milk thistle seed cakes: higher digestibility of protein, fat, Ca, P. The highest digestibility at 400 g/d. Significant difference in creatinine values. In the plasma, no flavonolignans traces were determined below the detection limit	[118]
Horses (equine athletes)	Milk thistle seed cakes	up to 400 g/d	16.6 g/d	56 d (horses exposed to heavy physical exercise)	Decrease of NEFA value was found after exercise indicating a higher utilization of NEFA during exercise. A faster return of cortisol to the resting values before exercise. Results demanded in equine athletes	[119]
in vitro/ex vivo **model hooves of slaughter horses**	Milk thistle and SIL	Milk thistle 1-1000 μg/mL SIL 100-250 μg/mL	Milk thistle (Sigma-Aldrich) SIL (Sigma-Aldrich)	24-48 h	Supported the prevention of laminitis: reduced endotoxin activity and inhibited LPS-induced effects on the lamellar tissue	[122]
			Silybin, Taxifolin			[123]

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in vitro/ex vivo **model equine neutrophils**	SIL+ Taxifolin+ Quercetin +Dehydrosilybin	100-10-1-0.1 µM	and Quercetin (Sigma-Aldrich) Dehydrosilybin (synthesized from Silybin)	several h	Modulate the oxidative response in the pathogenesis of laminitis: strong inhibition of neutrophils, and myeloperoxidase activity	
in vitro/ex vivo **model equine peripheral blood mononuclear cells**	Silibinin	5-10-50 µM	Silibinin (Sigma-Aldrich)	1 h, before stimulation with LPS for 4 h	Pharmacological approach useful in the treatment/prevention of inflammatory diseases such as laminitis: silibinin prevents the LPS induced increased levels of TNF-α, IL-1, IL-6, and IL-8	[124]

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