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Opportunities for sheep welfare improvement by silymarin additive – a review article

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Abstract

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Under grazing conditions, there are a number of stress factors that significantly impair the welfare of ewes and lambs - high and low temperatures, excessive solar radiation, poisonous plants, various endo- and ectoparasites, snake and insect bites, grazing infections, etc.

The purpose of this publication is to summarize and highlight the possibilities of using silymarin and *Silybum marianum* products as a dietary supplement to improve the welfare of sheep under pasture rearing.

A detailed analysis of numerous scientific articles related to the active ingredients, properties, and application of *Silybum marianum* products has been performed.

Data on the antioxidant, hepatoprotective and detoxifying effects of silymarin have been summarized. The dual hepatoprotective effect of silymarin has been emphasized particularly for its antioxidant action, associated with reducing the reactive oxygen species content and malondialdehyde properties, and for stimulating the activity of the antioxidant enzymes- glutathione peroxidase, superoxide, and heat shock protein 70 (HSP70).

The anti-stress effect of silymarin for lowering blood cortisol levels in animals is also briefly described, and pathways for silymarin detoxification of various toxins have been investigated.

As a result of these mechanisms, the silymarin effect on improving the live weight and milk yield of ruminants is explained.

Based on the review of the presented research, it can be concluded that silymarin and *Silybum marianum*-derivatives can be used successfully to improve sheep welfare under grazing conditions, due to their antioxidant, hepatoprotective, anti-stress, detoxifying activity, and to stimulate growth and milk production.

Keywords: silymarin; sheep welfare; antioxidant; anti-stress; detoxifying activity

Introduction

At present, sheep welfare in pasture and pasture-stable rearing has become an increasingly pertinent problem for agriculture, especially when it concerns the prevention and treatment of certain diseases. Under grazing conditions, there are a number of stress factors that significantly impair the welfare of ewes and lambs - high and low temperatures, excessive solar radiation, poisonous plants, various endo- and ectoparasites, snake and insect bites, grazing infections, etc.

The classic definition of animal welfare formulated by Broom (1986; 1996) sees it as a measurable physical condition that takes into account the animal's experience in dealing with the environment. "The difficulties encountered by the animal in this process and the inability of the animal to overcome these difficulties lead to a disturbance of its welfare" (Broom, 1996).

The essence of animal welfare is multifaceted and interpreted in several directions, such as "biological-functioning" (Broom, 1986; 1996; 2006; Moberg, 1996), "natural way of life" (Rollin, 1990; 1993) and "animal subjective experience" (Duncan & Dawkins, 1983; Dawkins, 1990; Duncan, 1996; Duncan & Fraser, 1997).

International animal welfare standards have been discussed for the past 15 years. Protocols were established to assess the welfare of various species of farm animals (European Livestock Welfare Quality® system (Botreau et al., 2007; WQ®, 2009, Veissier et al., 2011), including sheep - AWIN, 2015).

When considering opportunities to improve sheep welfare by silymarin additive, it is important to present the basic principles for maintaining sheep's well-being. These are Good Feeding, Good Housing, Good Health, and Appropriate Behavior. Silymarin supplementation would affect basically the principle - Good health. According to the AWIN Project, 2015, the principle of Good Health is based on the following criteria - Absence of injuries, Absence of disease, Absence of pain, and pain induced by management procedures. The essence of the possibilities for sheep welfare improvement is how silymarin will affect the Absence of disease- criterion, or how *Silybum marianum* products as a food supplement will affect the reduction of sheep diseases. This is directly related to the silymarin actions on the sheep organisms (Table 1).

In a summary of the possibilities for assessing sheep welfare by Dwyer & Bornett (2004), Turner & Dwyer, (2007), Dwyer (2009), Richmond et al. (2017) it was pointed out that the assessment of sheep welfare is related to certain indicators characteristic of the species health and behavior.

Animal behavior is provoked by various hormones, some of which related to positive emotions in sheep and others with arising stress factors (Johnson et al., 1992; Jensen et al., 1996; Terlouw et al., 1997, Dwyer & Bornett, 2004). In recent years, in order to characterize the healthy and stressful states in animal indicators of oxidative stress have been used, such as reactive oxygen species (ROS), malondialdehyde (MDA), glutathione peroxidase, superoxide dismutase,

Table 1. Opportunities to sheep welfare improvement by silymarin additive

Sheep welfare principles	Sheep welfare criteria	Silymarin action - authors
Good Feeding	Absence of prolonged hunger	
	Absence of prolonged thirst	
Good (Housing) Environment	Comfort around resting	
	Thermal comfort	
	Ease of movement	
	Absence of injuries	
		Antioxidant action - Oelrichs (1982), Thamsborg et al. (1996); Urbanczyk et al. (2002); Haddad et al. (2011), Sherif & Al-Gayyar (2013); Atanassof (2016); Karvellas et al. (2016); Alhidari et al. (2017); Jalali et al. (2017); Sahin et al. (2018); Khaleghipour et al. (2019); Saleemi et al.(2019); Stoev et al., (2019) Fanoudi et al. (2020); Khamisabadi, (2020); Pickova et al. (2020) ;
		Double hepatoprotective action - Gao et al. (2018); Stoev et al. (2019); Khamisabadi (2020).
Good Health	Absence of disease	Anti-stress action - Mahjoor & Dehghan (2008); Gong et al. (2015); Thakare et al. (2016); Khamisabadi (2020); Dockalova et al. (2021).
		Lactation-stimulating effect - Vojtisek et al. (1991), Grabowicz et al. (2004), Tedesco et al. (2002; 2004a; 2004 b); Khamisabadi, (2020);
		Growth-stimulating effect- Kim et al. (2013); Kosina et al. (2017); Grela et al. (2020).
	Absence of pain and pain induced by management procedures	
Appropriate behaviour	Expression of social behaviour	
	Expression of other behaviour	
	Good human-animal relationship	
	Positive emotional state	

catalase activity, etc. (Surai et al., 2016; 2017; Khamisabadi, 2020; Dockalova et al., 2021; Ivanov et al., 2021). In direct connection with sheep welfare, some productive indicators in ruminants have been studied - live weight, growth, milk yield, feed consumption, feed utilization, etc. (Vojtisek et al., 1991; Tedesco et al., 2002, 2004a, 2004b; Kim et al., 2013; Khamisabadi, 2020).

An important criterion for the level of sheep welfare is the state of their immune system (Minton & Blecha, 1990; Minton et al. 1992; 1995; Cockram et al., 1994; Sevi et al., 2001; Khamisabadi, 2020; Koynarski, 2020), as well as the condition of the liver.

Recently, as an alternative to the excessive use of antibiotics and in order to improve the health and well-being of sheep, plant products exhibiting antioxidant, anti-stress, hepatoprotective action such as silymarin have been used.

The purpose of this publication is to summarize and highlight the possibilities of using silymarin and *Silybum marianum* products as a dietary supplement to improve the welfare of sheep under pasture rearing.

Materials and Methods

To achieve the aims of this review, we have made a theoretical analysis of official documents and reports from international organizations such as the Food and Agriculture Organization (FAO), and the official website of the European Commission in connection with the use of silymarin in animal husbandry.

A detailed analysis of numerous scientific articles related to the active ingredients, properties, and application of Silybum marianum products has been performed. These research articles have been obtained from scientific databases such as PubMed (1966-June, 2021), EMBASE (1973-May, 2021), Research Gate, and Elsevier by keyword filtering.

Data on the antioxidant, hepatoprotective and detoxifying effects of silymarin have been summarized. The dual hepatoprotective effect of silymarin has been emphasized particularly for its antioxidant, associated with reducing the reactive oxygen species (ROS) content and malondialdehyde (MDA) properties and for stimulating the activity of the antioxidant enzymes glutathione peroxidase, superoxide, and heat shock protein 70 (HSP70).

The anti-stress effect of silymarin for lowering blood cortisol levels in animals is also briefly described, and pathways for silymarin detoxification of various toxins have been investigated.

As a result of these mechanisms, the effect of silymarin on improving live weight and milk yield of ruminants is explained.

Discussion

Nature and composition of silymarin

Silymarin is an extract from the seeds of the plant Milk thistle - *Silybum marianum* (L). Gaertn. (*Carduus marianus* L., *Asteraceae*), which have been used for more than 2000 years to treat liver and gallbladder diseases, including hepatitis and cirrhosis (Kroll et al., 2005; Atanassof, 2016).

The extract consists of about 65–80% silymarin (flavonolignan complex) and 20–35% fatty acids, including linoleic acid (Kroll et al., 2007). Silymarin is a complex mixture of polyphenolic molecules, including seven closely related flavonolignans (silibin A, silibin B, isosilibin A, isosilibin B, silychristine, isosilychristine, silidianin) and one flavonoid (taxifoline). Silibinin, a semi-purified silymarin fraction, is predominantly a mixture of 2 diastereoisomers, silibin A and silibin B, in a ratio of approximately 1: 1 (Kroll et al., 2007; Hogan et al., 2007). In addition to silybin, the extract contains significant amounts of other flavonoids, such as isosilybin, de-hydrosilybin, silychristin, silydianin, and several flavonoids, mainly taxifolin (Kroll et al., 2005). In addition, *S. marianum* is rich in 3-deoxyflavonoids.

In addition to its wide application in human medicine, silymarin is used as a dietary supplement in feeding farm animals, for treatment of parasitosis, and against the harmful effects of toxins in the feed of cattle (Tedesco et al., 2002; 2004), in sheep (Thamsborg et al., 1996), in pigs (Urbanczyk et al., 2003), in chickens (Gaweł et al., 2003; Tedesco et al., 2004;), in turkeys (Gaweł et al., 2003), in quails (Sakamoto et al., 2018), in carp (Atanasov, 2016), in grass carp (Xiao et al., 2016; 2017), and in rainbow trout (Ahmadi et al. , 2012; Banaee et al., 2011).

Beneficial action of silymarin for improving sheep welfare

Antioxidant action and double hepatoprotective action of silymarin

The strong antioxidant effect of silymarin against total oxidative stress at the beginning of lactation in sheep has been studied by Khamisabadi, 2020 who explores the possibilities for improving milk yield in Sanjabi ewes in the postpartum period with the addition of silymarin at doses of 2 g/kg feed for 15 days after lambing.

The author has found a significant reduction in the content of reactive oxygen species (ROS) and malondialdehyde (MDA) ($P<0.05$) in experimental sheep compared to controls. In addition, he reports an increased activity of endogenous liver antioxidant enzymes - glutathione peroxidase, superoxide dismutase, and catalase.

Similar data on the effect of silymarin on reducing the production of free radicals and ROS in rats, humans, broiler chickens have been presented by Haddad et al. (2011), Sherif & Al-Gayyar (2013) Khaleghipour et al. (2019). Silymarin action is associated with a reduction in lipid peroxidation and MDA levels in the blood and tissues and, thus, with a reduction of high levels of free radicals, lipid peroxidation, and protein carbonylation, leading to DNA chain damage and pathology in the body (Stone et al., 2010).

In addition, in a QRT-PCR analysis, Khamisabadi (2020), has found that silymarin reduces the expression of the HSP 70 protein shock protein gene in the blood serum of sheep (reducing HSP 70 mRNA levels) in the postpartum period in sheep. Silymarin has a hepatoprotective and nephroprotective effect in broiler chickens exposed to ochratoxin A-(OTA) (Gao et al., 2018; Stoev et al., 2019).

This is the mechanism of the antioxidant action of silymarin in animals. Silymarin is an effective strategy to reduce oxidative stress observed at the beginning of lactation in ewes, which is due to both the hepatoprotective and antioxidant effects of silymarin extracts (Haddad et al., 2011; Sherif & Al-Gayyar, 2013; Gao et al., 2018; Khaleghipour et al., 2019; Stoev et al., 2019; Khamisabadi, 2020).

The addition of silymarin, therefore, can reduce oxidative stress and heat shock protein activity and thus improve the well-being of sheep in the puerperium.

Anti-stress effect of silymarin

An important positive effect of silymarin to improve sheep welfare is its anti-stress effect, manifested by a reduction in cortisol levels in mammals.

Khamisabadi (2020) has explored the possibilities to improve the health of ewes in the postpartum period, with the addition of silymarin in doses of 2 g/kg of feed. The author suggests that by compensating for common oxidative stress in sheep at the beginning of lactation and reducing lipid peroxidation, silymarin is able to reduce cortisol levels. Therefore, future studies are recommended to elucidate the exact interactions between silymarin as an herbal antioxidant therapy and glucocorticoid secretion in animal models (Surai et al., 2016; 2017).

The anti-stress effect of *Silybum marianum* products has been confirmed by Dockalova et al. (2021) in experiments with horses. Cortisol released in horses is associated with the duration and intensity of training (Hodgson, 2014). Dockalova et al. (2021) have tested the effect of Milk thistle seed leaves (*Silybum marianum*) on the diet of horses subjected to heavy exercise (regular combined riding training) for 56 days at a dose of up to 400 g/day in normal doses of feed.

At the end of the treatment, one hour after completing the exercise scheme, the authors have found significantly lower cortisol levels (3.20 ± 0.32 μg/100 mL) in silymarin-treated horses compared to the control group (b 4.40 ± 0.39 l μg)/100 mL).

Similar data on the reducing effect of silymarin on stress-induced cortisol (Gong et al., 2015) in rats have been presented by Mahjoor and Dehghan (2008). The authors have performed an experiment on pregnant female Wistar rats exposed to stress as a result of a reduced diet (50% of normal food intake) and treated with silymarin at doses of 150, 200, 400 mg/kg. Serum cortisol decreases in rats with silymarin supplements at doses of 200, 400 mg/kg. The results of the study show that the addition of 200 mg/kg silymarin in Wistar rats exposed to acute stress is the most appropriate dose to achieve metabolic benefits. Silymarin has a positive effect on lipid metabolism and reduced serum concentrations of cortisol, triglycerides, and cholesterol under dietary restriction conditions.

Similar data for the reduction of corticosterone levels in mice exposed to acute stress due to silymarin activity have been presented by Thakare et al. (2016).

Antitoxic effects of silymarin

One of the most common applications of *S. marianum* and its main component - silymarin is their detoxifying function in intoxications with various toxic agents: biological and chemical toxins (Karvellas et al., 2016; Sahin et al., 2018; Fanoudi et al., 2020; Pickova et al., 2020). Silymarin can be used as an antidote or protective agent against chemical poisons - metals (Jalali et al., 2017; Saleemi et al., 2019), fluorides, pesticides, cardiotoxins, neurotoxins, hepatotoxins, and nephrotoxins (Karvellas et al., 2016; Sahin et al., 2018; Fanoudi et al., 2020; Pickova et al., 2020), and against biological venomous agents - snake and scorpion venom, bacterial toxins and xenobiotic mycotoxins (Fanoudi et al., 2020; Pickova et al., 2020).

Fanoudi et al. (2020) have found that the main protective effects of silymarin are due to several main mechanisms - radical removal, antioxidant, chelating, antiapoptotic properties, and regulation of inflammatory reactions.

In addition, a number of authors - Jalali et al. (2017); Saleemi et al. (2019); Fanoudi et al. (2020) have found the following mechanism of detoxification of silymarin against heavy metals: heavy metals show their toxicity through the production of free radicals, and silymarin is a proven radical scavenger. In addition, silymarin is a chelator that binds heavy metals to complex compounds and removes them from the body (Jalali et al., 2017; Saleemi et al., 2019; Fanoudi et al., 2020).

Silymarin also has an antitoxic effect in poisoning resulting from parasitosis in sheep.

Oelrichs (1982), Thamsborg et al. (1996) and Atanassof (2016) have found that silymarin can be used successfully in sheep parasitosis. One of its interesting applications is for the treatment of a disease in sheep caused by the larval forms of *Arge pullata*, occurring with massive necrosis of the liver and degeneration of the renal tubules. Ruminants ingest the larval forms of *Arge pullata* or *Lophyrotoma interrupta*, resulting in intoxication caused by toxic substances (octapeptide lophyrotomin). These are substances structurally similar to the toxic cyclic peptides in *Amanita* fungi. Silymarin treatment can be successfully administered as an antitoxic agent in sheep (Oelrichs, 1982; Thamsborg et al., 1996, Urbanczyk et al., 2002; Atanassof, 2016).

The protective effect of silymarin against the toxic effects of a number of mycotoxins is particularly relevant. Alhidari et al. (2017), demonstrate the beneficial effect of silymarin on the reduction of feed consumption and growth in broilers intoxicated with aflatoxin B1- (AFB1). Silymarin prevents immune suppression and has a hepatoprotective and nephroprotective effect in broiler chickens exposed to ochratoxin A- (OTA) (Stoev et al., 2019). In addition, the cytoprotective activity of silymarin against OTA, fumonisin B1 (FB1), and deoxynivalenol (DON) in porcine renal-15 cells (PC-15) have been established (Stoev et al., 2019). In addition, silymarin has a protective effect of on zearalenone-induced (ZEA) liver damage and reproductive toxicity in rats (Gao et al., 2018).

Stimulating effect of silymarin on ruminant growth and lactation

It has been reported that silymarin has a positive effect on the milk yield of ruminants. Data on the stimulatory effect of silymarin at doses of 2 g/kg feed on milk secretion and increase in total serum protein levels in ewes in the puerperal period are reported by Khamisabadi (2020). Similar, though less conclusive, data on lactogenic action of silymarin in goats are published by Forinash et al. (2012).

More definite results for the stimulatory effect of silymarin on the milk yield of cows are presented by Vojtisek et al. (1991) with soon-calved cows whose milk high concentrations of acetone (> 7.9 mg/L milk) have been registered. For two weeks, the experimental cows were fed feed containing seeds of Milk thistle (*Silybum marianum*) at doses of 0.3 kg per head/day, containing 2.34% silibinin and silidianin). The authors found a significant reduction in the concentrations of acetone + acetoacetic acid ($P < 0.01$) in the milk and blood of treated cows and beta-hydroxybutyric acid in the blood ($P < 0.05$), as well as in the degree of ketonuria. The study found that the milk yield of silymarin-treated cows increased by 7.7% (in test 1) and by 3.4% (in test 2) compared to controls. This pattern was maintained in cows receiving *Silybum marianum* supplementation even two weeks after discontinuation of the diet. Similar results for the positive effect of Milk thistle in soon-calved cows are presented by Grabowicz et al. (2004).

Furthermore, long-term studies by Tedesco et al. (2002, 2004a, 2004b) investigated the effect of the plant at a dose of 10 g of pure silymarin as an aqueous suspension for oral administration in cows, 10 days before the expected birth to 15 days after it. In the postpartum period, cows are predisposed to subclinical fatty liver disease. As a result of the silymarin treatment, higher milk yield was registered in experimental animals during the whole lactation period, and the peak of milk yield in the treated cows was one week earlier than the control group. Silymarin was also beneficial for lactation itself and for the live weight of treated animals after birth and during puerperium. No silymarin residues were found in the colostrum or in all other milk samples. Blood and milk parameters in the treatment of animals did not show adverse effects of the treatment.

Data on the stimulating effect of silymarin on the performance of ruminants were also presented by Kim et al. (2013) who investigated the effect of herbal plant supplements, including silymarin, on blood metabolites and carcass characteristics during the late feeding period in castrated male Hanu cattle. After 6 months of dietary supplementation, the author found a decrease in alanine aminotransferase activity (an important liver enzyme) and an increase in calf growth in the silymarin group compared to the control group. Therefore, silymarin could be introduced as an effective, harmless dietary supplement, as an alternative to dietary antibiotics, stimulating productivity during the late feeding stage of male castrated cattle of the genus Hanu.

Additional data to stimulate growth in rabbits and pigs are presented by Kosina et al. (2017) and Grela et al. (2020).

The above evidence confirms the positive silymarin effect on ruminant welfare.

Conclusion

Based on the review of the presented research, it can be concluded that silymarin and *Silybum marianum*-derivatives can be used successfully to improve sheep welfare under grazing conditions, due to its antioxidant, hepatoprotective, anti-stress, detoxifying activity, and to stimulate growth and milk production. Research in this direction must be continued.

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