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ВЕТЕРИНАРНОМЕДИЦИНСКИ ФАКУЛТЕТ

OPPORTUNITIES FOR SHEEP WELFARE IMPROVING BY SILIMARIN ADDITIVES - REVIEW

Assoc. Prof. Nadya Bozakova
and Prof. Veselin Ivanov

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Ministry of Education and Science
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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 sheep and lambs - high and low temperatures, excessive solar radiation, poisonous plants, various endo- and ectoparasites, poisoning by snakes, and insects, grazing infections, etc.

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INTRODUCTION

The classic definition of animal welfare formulated by Broom (1986) and Broom (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; Broom, 1996; Moberg, 1996, Broom, 2006), "natural way of life" (Rollin, 1990 and Rollin, 1999) and “animal subjective experience” (Duncan & Dawkins, 1983; Duncan, 1996; Duncanl & Fraser, 1997; Dawkins, 1990).

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INTRODUCTION

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 (WQ®, 2009, Botreau et al., 2007; Veissier et al., 2011), including the welfare of sheep - AWIN, 2015

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

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.

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THE PURPOSE OF THE STUDY

The purpose of this publication was 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.

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 2,000 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.

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MATERIALS AND METHODS

To achieve the aims of this review, we have made a theoretical analysis of official documents and reports from international organizations - Food and Agriculture Organization (FAO), 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 was 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 anti-stress, and detoxifying effects of silymarin have been summarized. The anti-stress effect of silymarin for lowering blood cortisol levels in animals is also briefly described, and pathways for detoxification of various toxins by silymarin have been investigated.

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

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DISCUSSION

Antioxidant action and double hepatoprotective action of silymarin

The strong antioxidant effect of silymarinat at doses of 2 g / kg feed for 15 days, against total oxidative stress at the beginning of lactation in sheep has been studied by Khamisabadi (2020). 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.

In addition, in a QRT-PCR analysis, the same author, has found that silymarin reduces the expression of the heat shock protein (HSP) 70-gene in the blood serum of sheep (reducing HSP 70 mRNA levels) in the postpartum period in sheep.

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

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.

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DISCUSSION

Anti-stress effect of silymarin

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

Khamisabadi, 2020 had 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 authors suggested 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.

The anti-stress effect of *Silybum marianum* products was proven by Dockalova at all. (2021) in experiments with horses. Dockalova at all. (2021) have tested the effect of Milk thistle seed loaves (*Silybum marianum*) on the diet of horses subjected to heavy exercise (regular combined riding training) for 56 days at a dose up to 400 g/day (in normal doses of feed).

At the end of treatment, one hour after cessation of exercise, 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).

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DISCUSSION

Anti-stress effect of silymarin

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.

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).

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DISCUSSION

Antitoxic effect 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; Pickova et al, 2020; Fanoudi 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; Pickova et al., 2020; Fanoudi et al., 2020) and against biological venomous agents - snake and scorpion venom, bacterial toxins and xenobiotic mycotoxins (Fanoudi at all, 2020; Pickova at all, 2020.

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DISCUSSION

Antitoxic effect of silymarin

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.

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 toxins of the larval forms of Arge pullata, occurring with massive necrosis of the liver and degeneration of the renal tubules.

Silymarin treatment can be successfully administered as an antitoxic agent in sheep (Atanasov 2016, Oelrichs, 1982; Thamsborg et al., 1996, Urbanczyk et al., 2002).

The protective effect of silymarin against the toxic effects of a number of mycotoxins is particularly relevant.

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DISCUSSION

Stimulating effect of silymarin on ruminant growth and lactation

Data on the stimulatory effect of silymarin on milk secretion and increase in total serum protein levels in ewes in the puerperal period are reported by Khamisabadi H., 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 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.

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, 2004 b 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.

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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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REFERENCES

1. **Ahmadi,** K., M. Banaee, A. Vosoghei, A. Mirvaghefei, B. Ataeimehr, 2012. Evaluation of the immunomodulatory effects of silymarin extract (Silybum marianum) on some immune parameters of rainbow trout (Oncorhynchus mykiss) (Actinopterygii: Salmoniformes: Salmonidae). Acta Ichthyol. Piscat. 42(2): 113-120, 2012
2. **Alhidary**, I.A.; Rehman, Z.; Khan, R.U.; Tahir, M. Anti-aflatoxin activities of milk thistle (*Silybum marianum*) in broiler. *Worlds Poult. Sci. J.* 2017, *73*, 559–566.
3. **Atanassof** Al., 2016. Study of the influence of the preparation Vitasil on the diet of carp (Cyprinus carpio) grown in a recirculation system. Dissertation for the award of educational and scientific degree Doctor, Stara Zagora, 2016, BG
4. **AWIN,** 2015. AWIN welfare assessment protocol for sheep. DOI: 10.13130/ AWIN _SHEEP_2015
5. **Banaee**, M., A. Sureda, M. Ali Reza, G. Reza Rafei, 2011. Effects of longterm silymarin oral supplementation on the blood biochemical profile of rainbow trout (Oncorhynchus mykiss). Fish Physiol Biochem. 37(4): 885-896, 2011
6. **Botreau, R., Veissier, I., Butterworth, A., Bracke, M.B.M. & Keeling, L.J.** (2007). Definition of criteria for overall assessment of animal welfare. *Animal Welfare*, *16*, 225–228.
7. **Bozakova, 2004.** Comparative studies and evaluation of welfare of turkey broilers on different floor type, PhD Thesis, Stara Zagora.
8. **Broom D.M.** (1986). Indicators of poor welfare. *British Veterinary Journal*, *142*(6), 524–526.
9. **Broom, D.M.** (1996). Animal Welfare Defined in Terms of Attempts to Cope with the Environment. *Acta Agriculturae Scandinavica, Section A, 27*, 22–28. 7.
10. **Broom,** D.M. 2006. Behaviour and welfare in relation to pathology. Appl. Anim. Behav. 2 Sci., 97, 71-83.

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REFERENCES

11. Dawkins M.S., 1998. Evolution and animal welfare, Quarterly Review of Biology, 73: 305-328.

12. Dockalova H, Zeman L, Horky P, 2021. Influence of Milk Thistle (*Silybum marianum*) Seed Cakes on Biochemical Values of Equine Plasma Subjected to Physical Exertion. Animals (Basel). 2021 Jan 16;11(1):210. doi: 10.3390/ani11010210.

13. Duncan I., 1996. Animal welfare defined in terms of feelings, Acta Agriculturae Scandinavica, Section A, Animal Science, Supplement, 27: 29-35

14. Duncan, I.J.H. & Dawkins, M.S. (1983). The problem of assessing 'well-being' and 'suffering' in farm animals. In Indicators relevant to farm animal welfare (D. Smidt, ed.), Martinus Nijhoff, The Hague, 13-24.

15. Duncan, I.J.H. & Fraser, D. (1997). Understanding animal welfare. In Animal welfare (M.C. Appleby and B.O. Hughes, eds), CAB International, Wallingford, 19-31.

16. Dwyer CM and HLI Bornett, 2004. Chronic stress in sheep: assessment tools and their use in different management conditions, *Animal Welfare* 2004, 13, 293-304.

17. Dwyer, C.M., 2009. Welfare of sheep: Providing for welfare in an extensive environment. *Small Ruminant Res.* Volume 86, Issues 1–3, October *2009*, Pages 14-21 (2009), doi:10.1016/j.smallrumres.2009.09.010

18. Fanoudi Sahar , Mohaddeseh Sadat Alavi , Gholamreza Karimi , Hossein Hosseinzadeh, 2020. Milk thistle (*Silybum Marianum*) as an antidote or a protective agent against natural or chemical toxicities: a review. Drug Chem Toxicol, 2020 May;43(3):240-254. doi: 10.1080/01480545.2018.1485687.

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REFERENCES

19. Forinash AB, Yancey AM, Barnes KN, Myles TD, 2012. The use of galactogogues in the breastfeeding mother - Review. Ann Pharmacother. 2012 Oct; 46 (10):1392-404. doi: 10.1345/aph.1R167.

20. Gao, X.; Xiao, Z.-H.; Liu, M.; Zhang, N.-Y.; Khalil, M.M.; Gu, C.-Q.; Qi, D.-S.; Sun, L.-H. Dietary silymarin supplementation alleviates zearalenone-induced hepatotoxicity and reproductive toxicity in rats. *J. Nutr.* 2018, *148*, 1209–1216.

21. Gaweł, A., B. Kotoński, J. Madej, M. Mazurkiewicz, 2003. Effect of silimarin on chicken and turkey broilers' rearing and the production indices of reproduction hen flocks. Medycyna Wet. 59(6): 517520, 2003

22. Gong S, Miao YL, Jiao GZ, et al. Dynamics and correlation of serum cortisol and corticosterone under different physiological or stressful conditions in mice. *PLoS One*. 2015;10(2):e0117503. Published 2015 Feb 20. doi:10.1371/journal.pone.0117503

23. Grabowicz, M., P. Dorszewski, P. Szterk, J. Mikołajczak, J. Piłat (2004). Influence of whole crop milk thistle silage on cows' metabolism in a transition period. Medycyna Wet. 60(7): 759762.

24. Grela, E.R.; Świątkiewicz, M.; Florek, M.; Wojtaszewska, I. Impact of milk thistle (*Silybum marianum* L.) seeds in fattener diets on pig performance and carcass traits and fatty acid profile and cholesterol of meat, backfat and liver. *Livest. Sci.* 2020, *239*, 104180.

25. Haddad Y, Vallerand D, Brault A, Haddad PS. Antioxidant and hepatoprotective effects of silibinin in a rat model of nonalcoholic steatohepatitis. Evid-Based Compl Alt. 2011; 2011.

26. Hogan, Fawn S.; Krishnegowda, Naveen K.; Mikhailova, Margarita; Kahlenberg, Morton S. (2007). "Flavonoid, Silibinin, Inhibits Proliferation and Promotes Cell-Cycle Arrest of Human Colon Cancer". *Journal of Surgical Research*. 143 (1): 58–65. doi:10.1016/j.jss.2007.03.080

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REFERENCES

27. Ivanov V., S. Dyankova, M. Pavlova, 2021. Comparative study of the antioxidant properties of silymarin and Milk thistle extracts. Trakia Journal of Sciences, No 1, pp 1-6, 2021. http://www.uni-sz.bg doi:10.15547/tjs.2021.01.001

28. Jensen KH, Hansen SW and Pedersen LJ 1996 The effect of long term stress on the hypothalamic–pituitary–adrenocortical axis and the role of the stressor. *Acta Agriculturae Scandinavia, Section A, Animal Science 27*: 40-45 (Suppl).

29. Johnson EO, Kamilaris TC, Chrousos GP and Gold PW 1992 Mechanisms of stress: a dynamic overview of hormonal and behavioural homeostasis. *Neuroscience and Biobehavioral Reviews*

30. Karvellas CJ, Tillman H, Leung AA, Lee WM, Schilsky ML, Hameed B, Stravitz RT, McGuire BM, Fix OK; United States Acute Liver Failure Study Group. 2016. Acute liver injury and acute liver failure from mushroom poisoning in North America.Liver Int. 2016 Jul;36(7):1043-50. doi: 10.1111/liv.13080. Epub 2016 Mar 4. Clinical Trial.

31. Khaleghipour B, Khosravinia H, Toghiyani M, Azarfar A. Effects of silymarin on productive performance, liver function and serum biochemical profile in broiler Japanese quail challenged with dietary aflatoxins. Ital J Anim Sci. 2019; 18(1): 564-573.

32. Khamisabadi, H. Effects of Silymarin on milk production, liver enzymes, oxidative status and HSP70 gene expression in postparturient Sanjabi ewes. *Cell. Mol. Biol.* 2020, *66*, 76–81.

33. Kim DH, Kim KH, Nam IS, Lee SS, Choi CW, Kim WY, Kwon EG, Lee KY, Lee MJ, Oh YK. 2013. Effect of indigenous herbs on growth, blood metabolites and carcass characteristics in the late fattening period of hanwoo steers.Asian-Australas J Anim Sci. 2013 Nov;26(11):1562-8. doi: 10.5713/ajas.2013.13132.

34. Kosina, P.; Dokoupilová, A.; Janda, K.; Sládková, K.; Silberová, P.; Pivodová, V.; Ulrichová, J. Effect of *Silybum marianum* fruit constituents on the health status of rabbits in repeated 42-day fattening experiment. *Anim. Feed Sci. Technol.* 2017, *223*, 128–140.

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REFERENCES

35. Koynarski, Tsvetoslav. 2020. “Prp Gene Polymorphism and Its Influence on Some Productive Traits of Sheep Breeds Reared in Bulgaria.” Bulgarian Journal of Veterinary Medicine 23 (2): 170

36. Kroll, D. J.; Shaw, H. S.; Oberlies, N. H. (2007). Milk Thistle Nomenclature: Why It Matters in Cancer Research and Pharmacokinetic Studies". *Integrative Cancer Therapies*. 6 (2): 110–9. doi:10.1177/1534735407301825

37. Kroll, Vl., Walterová. D. Silybin and silymarin- new effect and aplications. Biomed. Papers 149(1), 29–41 (2005).

38. Ledur, P.C.; Santurio, J.M. Cytoprotective effects of curcumin and silymarin on PK-15 cells exposed to ochratoxin A, fumonisin B1 and deoxynivalenol. *Toxicon* 2020, *185*, 97–103.

39. Mahjoor AA, Dehghan A., 2008. Effect of silymarin on metabolic factors of food-restricted over conditioned Wistar rats. Pak J Biol Sci. 2008 Jul 15;11(14):1835-9. doi: 10.3923/pjbs.2008.1835.1839.

40. Oelrichs, P. 1982. Sawfly poisoning of cattle. Queensland Agricultural Journal. 108: 110112, 1982

41. Pickova Darina, Vladimir Ostry, Jakub Toman , Frantisek Malir, 2020. Presence of Mycotoxins in Milk Thistle (*Silybum marianum*) Food Supplements: A Review, Toxins (Basel), 2020 Dec 8;12(12):782, doi: 10.3390/toxins12120782.

42. Richmond S.E., Francoise Wemelsfelder, Ina Beltran de Heredia, Roberto Ruiz , Elisabetta Canali and Cathy M. Dwyer, 2017. Evaluation of Animal-Based Indicators to Be Used in a Welfare Assessment Protocol for Sheep. *Front. Vet. Sci.* 4:210. doi: 10.3389/fvets.2017.00210

43. Sahin A, Arici MA, Yilmaz Y, Kalkan S, Durmus N, Ergur BU, Yakut Aksu I, Atabey N, Tuncok Y. A., 2018. Comparison of the Effectiveness of Silibinin and Resveratrol in Preventing Alpha-Amanitin-Induced Hepatotoxicity.Basic Clin Pharmacol Toxicol. 2018 Jun;122(6):633-642. doi: 10.1111/bcpt.12954. Epub 2018 Jan 24.

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REFERENCES

44. Sakamoto MI, Murakami AE, Fernandes AM, Ospina-Rojas IC, Nunes KC, Hirata AK, 2018. Performance and serum biochemical profile of Japanese quail supplemented with silymarin and contaminated with aflatoxin B1. Poult Sci. 2018 Jan 1;97(1):159-166. doi: 10.3382/ps/pex277.PMID: 29077959 Free article.

45. Saleemi MK, Tahir MW, Abbas RZ, Akhtar M, Ali A, Javed MT, Fatima Z, Zubair M, Bhatti SA, Zahoor Ul Hassan, 2019. Amelioration of toxicopathological effects of cadmium with silymarin and milk thistle in male Japanese quail (Coturnix japonica).Environ Sci Pollut Res Int. 2019 Jul;26(21):21371-21380. doi: 10.1007/s11356-019-05385-7. Epub 2019 May 23.

46. Sherif IO, Al-Gayyar MM. Antioxidant, anti-inflammatory and hepatoprotective effects of silymarin on hepatic dysfunction induced by sodium nitrite. Eur Cytokine Netw. 2013; 24(3): 114-121.

47. Stoev, S.D.; Njobeh, P.; Zarkov, I.; Mircheva, T.; Zapryanova, D.; Denev, S.; Dimitrova, B. Selected herbal feed additives showing protective effects against ochratoxin A toxicosis in broiler chicks. *World Mycotoxin J.* 2019, *12*, 257–268.

48. Stone CA, Kawai K, Kupka R, Fawzi WW. Role of selenium in HIV infection. Nutr Rev. 2010; 68(11): 671-681.

49. Surai P, Fisinin V. Antioxidant System Regulation: from Vitamins to Vitagenes. *Handbook of cholesterol*: Wageningen Academic Publishers; 2016: 1387-1393.

50. Surai PF, Kochish II. Antioxidant systems and vitagenes in poultry biology: heat shock proteins. *Heat Shock Proteins in Veterinary Medicine and Sciences*: Springer; 2017: 123-177.

51. Tedesco D., Domeneghini C., Sciannimanico D., Tameni M., Steidler S., Galletti S. 2004a. Silymarin, a possible hepatoprotector in dairy cows: biochemical and histological observations. Journal of Veterinary Medicine A-Physiology, Pathology, Clinical Medicine, 51, 85–89, 2004

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REFERENCES

52. Tedesco D., Tava A., Domeneghini C., Costa A., Sciannimanico D., Tameni M. 2002. Dietary inclusion of silymarin in periparturum dairy cows: Effects on milk productivity, plasma metabolites and liver tissue. Journal of Dairy Science, 80 (Suppl. 1), 244 (Abstr.), 2002

53. Tedesco D., Tava A., Galletti S., Tameni M., Varisco G., Costa A., Steidler S. 2004b. Effects of silymarin, a natural hepatoprotector, in periparturient dairy cows. Journal of Dairy Science, 87, 2239–2247, 2004

54. Tedesco, D., S. Steidler, S. Galletti, M. Tameni, O. Sonzogni, L. Ravarotto, (2004). Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B1 in broiler chicks. Poult Sci., 83: 1839-1843.

55. Tedesco, D.; Tava, A.; Galletti, S.; Tameni, M.; Varisco, G.; Costa, A.; Steidler, S., 2004. Effects of silymarin, a natural hepatoprotector, in periparturient Dairy Cows. *J. Dairy Sci.* 2004, *87*, 2239–2247.

56. Terlouw EMC, Schouten WGP and Ladewig J 1997. Physiology. In: Appleby MC and Hughes BO (eds) *Animal Welfare,* pp 143-158. CAB International: Wallingford, UK

57. Thakare, V.N.; Dhakane, V.D.; Patel, B.M. Potential antidepressant-like activity of silymarin in the acute restraint stress in mice: Modulation of corticosterone and oxidative stress response in cerebral cortex and hippocampus. Pharmacol. Rep. **2016**, 68, 1020–1027.

58. Thamsborg, S., J. Jorgensen, E. Brummerstedt, J. Bjerregard, 1996. Putative effect of silymarin on sawfly (arge pullata) ¬induced hepatotoxicosis in sheep. Vet. Hum. Toxicol. 38(2): 8991, 1996

59. Turner SP and CM Dwyer, 2007. Welfare assessment in extensive animal production systems:challenges and opportunities, Animal Welfare 2007, Volume 16, Number 2, May 2007, pp. 189-192(4) 16.

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Ministry of Education and Science
National Programme "European Scientific Networks"

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REFERENCES

60. Urbanczyk, J., E. Hanczakowska, M. Swiatkiewicz, 2002. Herb mixture as an antibiotic substitute in pig feeding. Medycyna Wet. 58(11): 887889, 2002

61. Veissier, I., Jensen, K.K., Botreau, R. & Sandøe, P. (2011). Highlighting ethical decisions underlying the scoring of animal welfare in the Welfare Quality scheme. *Animal Welfare*, *20*, 89–101.

62. Vojtisek, B., B. Hronova, J. Hamrik, B. Jankova (1991). Milk thistle in the feed of ketotic cows. Vet Med (Praha). 36(6): 32130.

63. Welfare Quality ® (2009). Welfare Quality ® assessment protocol for pigs (sows and piglets, growing and finishing pigs). Welfare Quality ® Consortium, Lelystad, Netherlands.

64. Xiao P., Yang Z., Sun J., TianJ., Zhang Z., Ji X.,Zhang B., Ye Y., Ji H., Yu E., Xie J., 2017. Silymarin inhibits adipogenesis in the adipocytes in grass carp Ctenopharyngodon idellus in vitro and in vivo.Fish Physiology and Biochemistry, 23 Jun 2017, 43(6):1487-1500
DOI: 10.1007/s10695-017-0387-7

65. XiaoP., Ji H., Ye Y., Zhang B., ChenY., TianJ., LiuP. Chen L., DuZhenyu, 2016. Dietary silymarin supplementation promotes growth performance and improves lipid metabolism and health status in grass carp (Ctenopharyngodon idellus) fed diets with elevated lipid levels.Fish Physiology and Biochemistry, 08 Sep 2016, 43(1):245-263.
DOI: 10.1007/s10695-016-0283-6