

Review

Anthocyanins in Dairy Cow Nutrition: A Review

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Abstract: Oxidative stress is a condition in dairy cows characterized by an imbalance of prooxidants and antioxidants, abnormally high levels of free radicals, or a decline in antioxidant defense mechanisms. Moreover, dairy cows are more inclined to oxidative stress status, which causes damage to the body and DNA damage resulting in a decline in antioxidant potential. Anthocyanins are effective antioxidants and free radical scavengers derived from fruit, purple corn, and other purple plants in nature. Many studies have shown that anthocyanins have favorable antioxidation effects in dairy cows and prevent lipid oxidation to improve milk quality. Accordingly, in the present paper, we mainly review (1) the definition of anthocyanins and the possible absorption and metabolic pathways; (2) the effect of anthocyanins on rumen fermentation parameters and methane production; (3) the mechanism by which anthocyanins enhance antioxidant potential and alleviate oxidative stress by scavenging free radicals and regulating signalling pathways; and (4) the effect of anthocyanins on milk production and milk lipid oxidation in dairy cows. This paper may provide an important rationale for the modulation of dairy cow health and productivity by anthocyanins in the future.

Keywords: anthocyanins; antioxidant activity; oxidant stress; milk oxidation; dairy cow



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1. Introduction

Oxidative stress (OS) occurs when there is an increase in oxidant production and free radical (FR) formation that exceeds ruminants' ability to neutralize and eliminate these reactive radical forms [1]. Dairy cows are inclined to OS status due to their intensive metabolic demands for maintenance and production, thereby resulting in various damages, including ketosis, respiratory acidosis, sepsis, and pneumonia [2]. As a result, many sources of OS induce the accumulation of numerous reactive oxygen species (ROS) in animal cells, and thus, OS has side effects on dry matter intake (DMI), growth performance, rumen fermentation, milk production, and inflammatory response [3,4]. One study showed that high-producing dairy ruminants can be optimized to a certain extent by the addition of diets with optimal levels of micronutrients with antioxidant capabilities [5].

Currently, new natural antioxidants are quite popular due to their safety for consumers. Natural antioxidants are important bioactive compounds that can delay ageing in organisms on the basis of their ability to scavenge FRs [6,7]. Consequently, these antioxidants play an important role in preventing oxidative injury through their ability to scavenge FRs that cause cellular damage [8]. For example, Xiao et al. [9] indicated that supplementation with vitamin E and Se in dairy cow diets might have a positive impact on the enhancement of antioxidant status and immune responses to enhance body health. Numerous studies have reported that flavonoid compounds (including anthocyanins) can reduce OS in ruminants [10,11].

Anthocyanins are large flavonoid compounds that are strong natural antioxidants and exist widely in the natural-plant and food fields [12,13]. Indeed, anthocyanins are components of plants that have strong antioxidant potential and can donate extra electrons to the FR and neutralize excessive electrons, alleviating OS status in animals and reducing oxidative damage to cells, tissues, proteins, cellular membranes, and mitochondria [14,15].

Moreover, anthocyanins are a powerful source of antioxidants and might improve ruminants' production by altering intracellular OS and changing ruminal fermentation [16]. Therefore, we review (1) the definition of anthocyanins and the possible absorption and metabolic pathways; (2) the effects of anthocyanins on rumen fermentation parameters and methane production; (3) the mechanism by which anthocyanins enhance antioxidant potential and alleviate oxidative stress by scavenging FRs and regulating signalling pathways; and (4) the effect of anthocyanins on milk production and the mechanism by which anthocyanins prevent milk lipid oxidation in dairy cows.

2. Definition of Anthocyanins

Anthocyanin is the general name applied to the glycosides of anthocyanidin chromophores, which are the origin of the red, violet, and blue colours found throughout the plant kingdom, such as the colours of petals, leaves, and fruits [17]. Anthocyanidins are the basic structures of anthocyanins (Figure 1; [18]). The anthocyanidins (or aglycons) consist of an aromatic ring [A] bonded to a heterocyclic ring [C] that contains oxygen, which is also bound to a third aromatic ring [B] by a carbon–carbon bond [19,20]. When the anthocyanidins are found in their glycoside form (bonded to a sugar moiety), they are known as anthocyanins [19,21]. To date, there are reports of more than 500 different anthocyanins and 23 anthocyanidins [22], of which the following six are the most common in vascular plants (Table 1): pelargonidin (Pel), peonidin (Peo), cyanidin (Cya), malvidin (Mal), petunidin (Pet), and delphinidin (Del) [18,23]. Moreover, the distributions of these six anthocyanidin classes in fruits and vegetables are 50% Cya, 12% Del, 12% Pel, 12% Peo, 7% Pet, and 7% Mal [21].

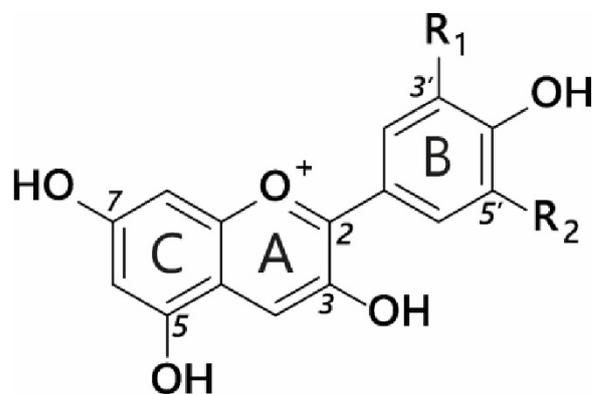


Figure 1. The structure of anthocyanins [18].

Table 1. Six most common anthocyanidins [18].

Anthocyanidin	R1	R2
Pelargonidin	H	H
Peonidin	OCH ₃	H
Cyanidin	H	OH
Malvidin	OCH ₃	OCH ₃
Petunidin	OH	OCH ₃
Delphinidin	OH	OH

There are a number of plants that have an abundance of anthocyanins, which can be used as feed additive for dairy cows. Taethaisong et al. [24] found that purple neem foliage had 132.89 mg/g Cya, 39.96 mg/g Del, 10.93 mg/g Pel, 9.49 mg/g Peo, 32.60 mg/g Pet, and 19.67 mg/g Mal. Suong et al. [25] showed that anthocyanin-rich black cane had 0.158 mg/g Cya, 0.080 mg/g Del, 0.080 mg/g Pel, and 0.393 mg/g Mal. Similarly, Tian et al. [26] found that purple corn pigment had 1.97 mg/g Cya, 0.59 mg/g Del, 0.045 mg/g Pel, and 0.0079 mg/g Pet. Anthocyanins have both antioxidant and anti-inflammatory properties

and are increasingly being studied for their effect on various health challenges ranging from cancer to aging, heart disease, and diabetes. Thus, anthocyanin-rich plants display a higher level of anthocyanin content than other sources, making this group of plants one of the most exciting new super feedstuffs at present.

There are an enormous variety of factors affecting the stability of anthocyanin, including pH, oxygen level, temperature, and light [27]. The pH value is the main factor affecting the degradation of anthocyanins in the gastrointestinal tract of dairy cows. Reyes and Cisneros-Zevallos [28] found that the degradation rate of anthocyanin increased with increasing pH values under the same external conditions. Consequently, anthocyanins can be stable in a low-pH environment. This is because anthocyanins can exist in three kinds of chemical equilibrium in acid aqueous solution, acid-base equilibrium, hydration equilibrium, and ring-chain tautomeric equilibrium [29]. Four kinds of structures are responsible for colour changes and stability/instability, including quinonoidal anhydrobase (A, blue), flavylium cation (AH^+ , red), carbinol pseudobase (B, colourless), and chalcone (C, colourless or light yellow). When $pH > 7$, the acid-base balance becomes the main cause of the anthocyanin proton transfer reaction from (AH^+) to (A) or the anthocyanin ionization from (AH^+) to (A^- or A^{2-}); A^- and A^{2-} are unstable compared to the AH^+ structure and thus more easily degraded to other products. When $pH < 7$, the above three chemical equilibria exist simultaneously, mainly due to the anthocyanin structure in this pH range [29].

3. Possible Absorption and Metabolic Pathways in Dairy Cows

Understanding the metabolism and bioavailability pathways of anthocyanins in the rumen in dairy cows is critically important for improvement in OS status. Dairy cows possess highly developed systems to maintain ruminal pH within a physiological range of approximately 5.5–7.0 [30]. As previously mentioned, anthocyanins are unstable under high pH conditions [29]. However, anthocyanins can also be stable in acidic aqueous solutions [31]. Furthermore, anthocyanins are phenolic compounds that may bind to the nutrients to be digested, inhibiting digestive enzymes and exerting antimicrobial effects [32,33]. Hence, the ruminal environment provides the necessary conditions for a rumen bypass of the anthocyanins. In our study, the anthocyanin-rich plants did not degrade in the ruminal fluid via the *in vitro* gas production technique [34], suggesting that the intestine may be the major place where ruminants absorb anthocyanins [35]. These previous observations led us to presume that ingested anthocyanin is probably absorbed and exhibits a functional effect in ruminant animals if the anthocyanin is not broken down in the rumen. This is because the digestion and absorption functions in the abomasum and intestines of ruminants are analogous to those in the alimentary canal of monogastric animals [36]. Thus, anthocyanins in plants might be maintained under a stable rumen condition in dairy cows. Anthocyanins can be absorbed into the body, but their bioavailability is very low in animals relative to the other flavonoid compounds [37]. The addition of anthocyanin-rich plants in the diet of lactating dairy ruminants (approximately 4.47 mg/kg body weight) resulted in anthocyanins being transferred to milk, increasing the concentrations of some individual anthocyanin components (Peo and cyanidin-3-glucoside) in milk [38]. He and Giusti [39] indicated that the daily requirement of anthocyanins for humans was approximately 2.5 mg/kg body weight. Therefore, these anthocyanin compositions in milk are very low, but they may be important for human health due to their unique antioxidant properties.

4. Effect of Anthocyanins on Rumen Fermentation and Methane Production

An excessively low or high ruminal fluid pH might result in negative effects on animal health [40]. The ingestion of an anthocyanin-rich diet results in a stable pH environment in the rumen by stimulating rumination and alkaline saliva production [41]. Anthocyanins could improve the ruminal fluid ammonium nitrogen concentration and thus improve the assimilation by ruminants of dietary amino acids [42]. One explanation could be that

anthocyanins can bind to dietary proteins and bypass the rumen to reach the small intestine for digestion and increase nitrogen utilization [43]. Volatile fatty acids (VFAs) function for energy storage, growth, reproduction, lactation, and other basic activities in ruminant animals [44]. The feeding of anthocyanin-rich plants or pigments could improve the total VFA concentration and individual VFA concentrations in ruminal fluid [45]. Specifically, anthocyanins consist of anthocyanidin and sugar(s), which are sugar structures that might participate in VFA production in dairy cows. Moreover, dietary anthocyanins in the ruminal microbiota may have a synergistic action, and thus, anthocyanins have effects on ruminal microbiota, modifying the gas production kinetics and VFA proportions and improving the acetate: propionate ratio [46].

The production of methane is the main cause of fermentation energy loss in ruminants. Reducing its production is very helpful to improve the energy efficiency of the diet and protect the environment. A high content of propionic acid in the rumen may lead to less methane production, indicating increased energy utilization; this is because a negative correlation exists between CH₄ production and propionate production because of their competition for hydrogen [47]. Interactions between anthocyanins and ruminal microorganisms occur, and hydrogen ions decrease because of the lower feed degradability and inhibition of fibre digestion in the presence of anthocyanins [48]. Anthocyanins can not only promote the flow of microbial proteins and reduce methane production in the gastrointestinal tract but can also combine with proteins to form complex nutrients and improve feed utilization efficiency and reduce methane emissions [49]. Moreover, anthocyanin compounds decrease methane production when added to ruminant diets by suppressing the growth and activity of methanogens, such as *Methanobrevibacter* and *Methanomicrobium*, responsible for *methanogenesis* [46]. Moate et al. [50] showed that the addition of anthocyanin-rich plants could play a role in CH₄ abatement because it can decrease CH₄ emissions and CH₄ yield by approximately 20% in lactating, multiparous Holstein-Friesian cows. Thus, the potential of anthocyanins to inhibit CH₄ production in dairy cows was as follows: (1) anthocyanins may reduce ruminal fluid methanogenesis, acting as H₂ sinks; (2) anthocyanins can reduce fibre digestibility in the rumen, resulting in lower methane production; and (3) anthocyanins might inhibit methanogens and hydrogen-producing microbe growth and activity [51,52].

5. Effect of Anthocyanins on Antioxidant Activity

Living organisms have a redox system, which functions to keep host dairy cows living in a healthy balance [53]. FRs are chemical species containing unpaired electrons that can increase the reactivity of atoms or molecules. FRs are highly reactive and unstable due to their unpaired electrons and can react locally by accepting or donating electrons to become more stable [54]. The reaction between a radical and a nonradical compound generally leads to the propagation of the radical chain reaction and to an increasing generation of new FRs [55]. Under normal conditions, superoxide anion (O²⁻) is converted into hydrogen peroxide (H₂O₂) by superoxide dismutase (SOD); H₂O₂ is then converted into H₂O by the activity of glutathione peroxidase (GPX) and catalase (CAT) in ruminants [56]. Reduction in peroxides is accompanied by oxidation of reduced glutathione (GSH), which can be regenerated from glutathione disulfide by reducing equivalents from NADPH, which is generated by the pentose monophosphate shunt. The resulting destruction of GSH increases the consumption of reducing equivalents, diverting glucose from important physiological pathways and competing with NADPH-dependent metabolic pathways in animals, such as energy metabolism, immunological functions, antioxidation capacity and calcium homeostasis [57]. Thus, FRs are necessary for the living state of cells and organisms, and in dairy cows, FRs at normal levels can protect normal function well [58]. For instance, some FRs, such as nitric oxide, O²⁻, and related ROS, mediate signalling processes in cells [59]. However, redox homeostasis could be unbalanced under stress conditions and extreme environments, resulting in the generation of radicals that exceed the needs of the body. OS occurs when there is a serious imbalance in any cell compartment between the production of ROS and antioxidant defence, leading to damage [1]. The overwhelming

FRs could lead to ageing, production diseases (e.g., mastitis, metritis, retention of foetal membranes, etc.) and other health problems in dairy cows [60].

High OS is common in organs and tissues with high metabolic and energy demands, including skeletal and heart muscle, the liver, and blood cells [61]. As energy demands exceed energy intake, dairy cows become metabolically challenged and enter a state of negative energy balance. This triggers catabolic pathways that, at the cellular level, increase the production of ROS, resulting in decreased performance production in the cows [62]. A considerable number of studies have shown that OS not only has a negative impact on DMI, milk yield and composition, and reproductive performance but also results in a decrease in economic benefits in dairy cows [63,64]. Bagchi et al. [65] demonstrated that grape seed anthocyanin extract is a more potent scavenger of oxygen FRs than vitamin C or E. They found that at a 100 mg/L concentration, grape seed anthocyanin extract exhibited 78–81% inhibition of O^{2-} and hydroxyl radicals. Anthocyanins, as a kind of antioxidant, can provide electrons to FRs, preventing them from oxidizing nearby cells to maintain the FR balance when host animals are in OS status, and they can improve antioxidant potentials in animals by enhancing hepatic Mn-SOD, Cu/Zn-SOD, and GPX mRNA expression [66]. Hence, anthocyanin has an active phenolic hydroxyl structure, which has a similar antioxidant mechanism to that of polyphenols, and the possible mechanism of improving antioxidant activity is as follows: (1) the phenolic hydroxyl group possesses the function of directly scavenging oxygen FRs through its own structure, improving the body's antioxidant-related enzyme activity to improve the body's antioxidant capacity; and (2) anthocyanin plants inhibit inflammatory processes in the mucosa of the intestine because of the promotion of intestinal microflora normalization, exclusion of pathogens, reduction in the permeability of the intestinal barrier, and promotion of the immune response and of antioxidant activity [67,68].

6. Effect of Anthocyanins on the Nrf2 and NF- κ B Signalling Pathways

It is well known that nuclear factor erythroid 2-related factor 2 (Nrf2) binds to Kelch-like ECH protein-1 (Keap1), a cytoskeleton-associated protein, within the cytoplasm under normal conditions [69]. However, some inducers from external electrophiles and oxidants have the potential to modify Keap1 cysteines, leading to the detachment of the Nrf2 DLG motif from Keap1. Anthocyanins are the glycosides of the polyhydroxy of the flavylum cation, which are a source of electrophiles and respond to activate the Nrf2 signalling pathway [70]. As Nrf2-activating agents, anthocyanins can directly modify sensor cysteines present in Keap1, activating Nrf2 and regulating phase II enzymes and antioxidant proteins NAD(P)H dehydrogenase, quinone 1 (NQO1), glutathione S-transferase, haem oxygenase-1, CAT, and SOD2 to relieve OS status in animals [71]. Thus, the feeding of an anthocyanin-rich diet could increase the expression of the SOD2, GPX1, and GPX2 genes in the mammary gland of dairy ruminants [72]. Hosoda et al. [73] suggested that lactating dairy cows receiving anthocyanin-rich corn showed a higher level of SOD activity. Similarly, the inclusion of anthocyanin-rich grape seed and grape marc meal extract can also improve the mRNA abundances of Nrf2 target genes (CAT, GPX3, microsomal glutathione S-transferase 3, NQO1, SOD1, UDP glucuronosyltransferase 1, and polypeptide A1) in the liver of Holstein cows [74].

OS results in large increases in reactive carbonyl compounds, with the appearance of carbonyls in the biosystem of ruminants. These excessive FRs can stimulate nuclear factor kappa beta (NF- κ B), tumour necrosis factor (TNF), interleukins, and cellular adhesion molecules and cause a series of inflammatory responses [75], subsequently leading to a drop in the antioxidant capacity of dairy cows and impairing mitochondrial function in the body of dairy cows [76]. I κ B kinase plays a very important role in the process of NF- κ B dimer activation through phosphorylation of inhibitory κ B, which is beneficial for inactivating NF- κ B nuclear transcription in the cytoplasm [77]. Additionally, NF- κ B is a major regulator of inflammatory responses; thus, some inflammatory stimuli induce TNF, chemokine, cytokine, heat shock transcription factor 1, and heat shock 70 kDa protein 1A (hspA1A) gene

expression in mitochondria mainly through activation of the NF- κ B signalling pathway [78]. As a consequence, NF- κ B is a nuclear transcription factor that participates in numerous signal transduction pathways in the process of inflammation [79].

Anthocyanins protect against inflammation by associating with altered inflammation-related gene expression and by limiting the expression of adhesion molecules [80]. Consequently, anthocyanins might be involved in I κ B kinase phosphorylation, regulating the inflammatory signalling pathway via inhibition of FR production and the expression of the NF- κ B transcription factor [81]. Gessner et al. [74] demonstrated that liver inflammation-related gene expression (C-reactive protein, haptoglobin, and TNF) tended to decrease in Holstein cows receiving 1% anthocyanin-rich grape seed and grape marc meal extract. Similarly, the feeding of polyphenol-rich feedstuffs could also downregulate heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa) in the liver, suggesting that anthocyanins can inhibit related inflammatory processes [82]. The postulated mechanism by which anthocyanins modulate the Nrf2 and NF- κ B signalling pathways in dairy cows is presented in Figure 2.

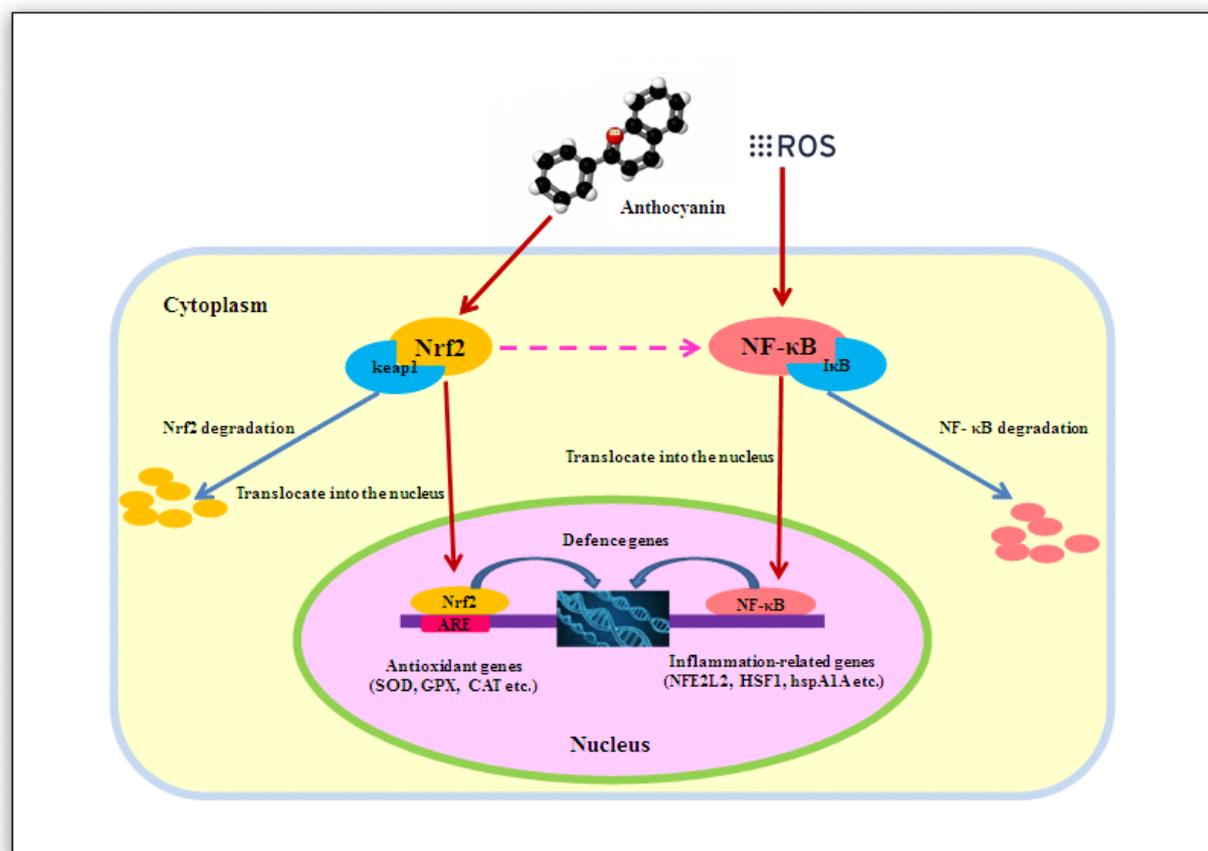


Figure 2. The postulated mechanism by which anthocyanins modulate the Nrf2 and NF- κ B signalling pathways in dairy cows. Nrf2 and Keap1 are bound together in the cytoplasm under normal conditions, whereas anthocyanins may lead to Nrf2 degradation to isolate Keap1. Next, Nrf2 can translocate into the nucleus and then bind to the antioxidant response element and activate expression of related antioxidant genes and detoxifying enzymes. Moreover, anthocyanins can remove FRs and indirectly inhibit the NF- κ B signalling pathway in dairy cows. ROS: reactive oxygen species; Nrf2: nuclear factor erythroid 2-related factor 2; NF- κ B: nuclear factor kappa beta; Keap1: Kelch-like ECH protein; I κ B: I κ B kinase; ARE: antioxidant response element; SOD: superoxide dismutase; GPX: glutathione peroxidase; CAT: catalase; HSF1: heat shock transcription factor 1; hspA1A: heat shock 70 kDa protein 1A.

7. Effect of Anthocyanins on Milk Production

High-producing lactating dairy cows are prone to being in OS status, reducing milk yield and negatively affecting milk composition [83]. Anthocyanins have a bitter taste; thus, their addition to ruminant diets may result in low DMI. However, recent studies have shown that DMI did not differ when anthocyanin-rich pigments or plants were included in ruminant diets, which might be due to the better palatability at lower inclusion levels [26,36]. Thus, supplementation with anthocyanins in the dairy cow diet would not decrease milk production. Matsuba et al. [84] found that Holstein dairy cows receiving anthocyanin-rich plants had increased milk yield (31.7 vs. 29.2 kg/d) and blood SOD concentrations (9333 vs. 8467 U/mL) compared with those fed a control diet. In addition, Matra et al. [85] showed that Holstein-Friesian crossbred cows receiving 400 g/d phenolic-rich dragon fruit peel pellets had improved milk yield and produced 3.5% fat corrected milk.

Interestingly, the potential mechanism for milk fat depression during OS is rumen fatty acid biohydrogenation—inhibiting *de novo* milk fat synthesis [86]. The current reigning “trans-fatty acid or biohydrogenation” theory of MFD suggests that specific intermediates of ruminal fatty acids—notably trans-10 and cis-12 conjugated linoleic acid (CLA)—will biohydrogenate, escape the rumen, and signal a decrease in lipogenic enzymes, causing a reduction in mammary gland milk fat synthesis [87]. As mentioned earlier, the feeding of anthocyanin-rich diets can potentially increase DMI to decrease negative energy balance, impacting rumen fermentation parameters, especially VFAs, to control the fatty acid biohydrogenation pathway in dairy cows. In our previous study, 16S rRNA sequencing assays showed that the inclusion of anthocyanin plants in the diet of ruminants had the ability to enhance antioxidant activity, improve ruminal fluid VFAs, and induce a shift in the structure and relative abundance of ruminal microbiota [43]. Anthocyanins may affect rumen microorganisms to prevent fatty acid biohydrogenation pathway changes by maintaining the growth of rumen microorganisms that produce trans-11 fatty acid isomers or inhibiting the growth and function of rumen microorganisms that produce trans-10 C18:1. Hence, anthocyanin compounds could alter the ruminal microorganisms, altering some steps of biohydrogenation [51]. Khonkhaeng et al. [88] showed that the feeding of anthocyanin-rich purple field corn residue improved milk fat CLA and arachidonic acid concentration and decreased CH₄ production compared to the control in dairy cows.

8. Effect of Anthocyanins on Milk Oxidation

Milk is rich in protein, vitamins, minerals, and enzymatic and nonenzymatic antioxidant components and is one of the most suitable livestock nutrition sources for humans [89,90]. However, UFAs oxidize lipid radicals, oxidize peroxy radicals with O₂, and then oxidize fatty acid hydroperoxide with hydrogen, thus negatively affecting milk antioxidant enzymes [91]. Oxidative deterioration of polyunsaturated lipids is one of the most important problems in food chemistry because lipid oxidation products cause undesirable flavours and lower the nutritional quality and safety of lipid-containing dairy foods [92]. Indeed, milk lipid oxidation not only affects milk quality and produces off-flavour compounds but also harms human health [91].

The general process of lipid peroxidation consists of three stages: initiation, propagation, and termination [93]. Many species can initiate the chain reaction of the radicals hydroxyl, alkoxyl, peroxy, superoxide, and peroxyxynitrite. As a consequence, an FR attracts a proton from a carbon of a fatty acyl side chain, leaving the remaining carbon radical accessible to molecular oxygen to form a lipid peroxy radical, which is also highly reactive, propagating the chain reaction further. As a result, PUFA molecules are transformed into conjugated dienes, peroxy radicals, and hydroperoxides, which undergo cleavage, mainly to aldehydes. More than 20 lipoperoxidation end-products were identified [94]; among the components of PUFA oxidative degradation products, the most frequently mentioned were acrolein, malondialdehyde (MDA), 4-hydroxyalkenals, and isoprostanes [95]. MDA results from the lipid peroxidation of polyunsaturated fatty acids, and the degree of lipid peroxidation in milk can be estimated by the amount of MDA, which is a marker of OS [96]. In short,

different forms of lipoperoxides can be formed in milk, such as fatty acid hydroperoxides and phospholipid hydroperoxides; these can be reduced by various GPXs. H_2O_2 can be produced by sulfhydryl oxidase and xanthine oxidase, and xanthine oxidase may also give rise to superoxide. SOD catalyses the formation of H_2O_2 from superoxide. The conversion of H_2O_2 into hydroxyl radicals can be controlled by the availability of iron ions, which is influenced by lactoferrin and transferrin. Moreover, H_2O_2 can be consumed in reactions catalysed by lactoperoxidase, CAT, and GPX [91].

Anthocyanins are important bioactive substances and antioxidants, and thus anthocyanin-rich milk is a good source of antioxidants and is popular among consumers [97]. More specifically, the addition of anthocyanins from plants seems to be an effective method for retarding lipid oxidation because it maintains UFA content in milk during storage [98]. The inclusion of anthocyanin-extracted residue could reduce meat oxidation and increase PUFA in meat in dairy cattle [99]. Indeed, anthocyanins can provide H atoms to peroxy radicals and inhibit lipid oxidation by chain radical termination, which has the ability to prevent lipid oxidation, enhance antioxidant activity, maintain volatile compounds, and increase the sensory scores in milk [97]. The postulated mechanism of anthocyanins delaying the oxidation of milk fat was as follows: (1) anthocyanins, as a source of flavonoids, can inhibit lipid peroxidation by acting as strong O^{2-} scavengers and O_2 quenchers and react with peroxy radicals, which are responsible for radical chain reactions during lipid peroxidation [100]; (2) they can act as H-atom donors to peroxy radicals, thus inhibiting the oxidation of fatty acids by chain radical termination [101]; and (3) anthocyanins show high antioxidant function and increase milk antioxidant enzyme levels, inhibiting the auto-oxidation of fatty acids [102]. In view of the reactivity of flavonoids with peroxy radicals and the noncompetitive inhibitory nature of the anthocyanins observed in this study, anthocyanins might interfere with or quench fatty radicals and thus terminate the radical chain reaction necessary for the propagation of lipid peroxidation [102].

9. Conclusions

In summary, anthocyanins can be used as natural antioxidants in dairy cows (Figure 3) because (1) anthocyanins can improve rumen fermentation parameters and reduce methane production; (2) anthocyanins can neutralize and decrease FR and ROS production to enhance antioxidant activity; (3) anthocyanins can activate the Nrf2 signalling pathway and inhibit the NF- κ B signalling pathway, modulating antioxidant and inflammation-related gene expression; and (4) anthocyanin-rich plants have the potential to transfer anthocyanins to milk to increase milk quality and inhibit lipid oxidation, enhancing consumer health. However, the absorption and metabolism pathways of anthocyanins in ruminants are still unclear, and various issues require further research and discussion. In addition, anthocyanin-rich plants are the main research feedstuff examined in the present study, which not only contain anthocyanin compounds but also have other natural antioxidants, such as flavonoids, polyphenols, and vitamins. Consequently, further *in vivo* studies of ruminants are encouraged to determine the degradation pathways of absorption and excretion of individual anthocyanin components and the mechanism by which anthocyanins alleviate OS status and enhance antioxidant potential.

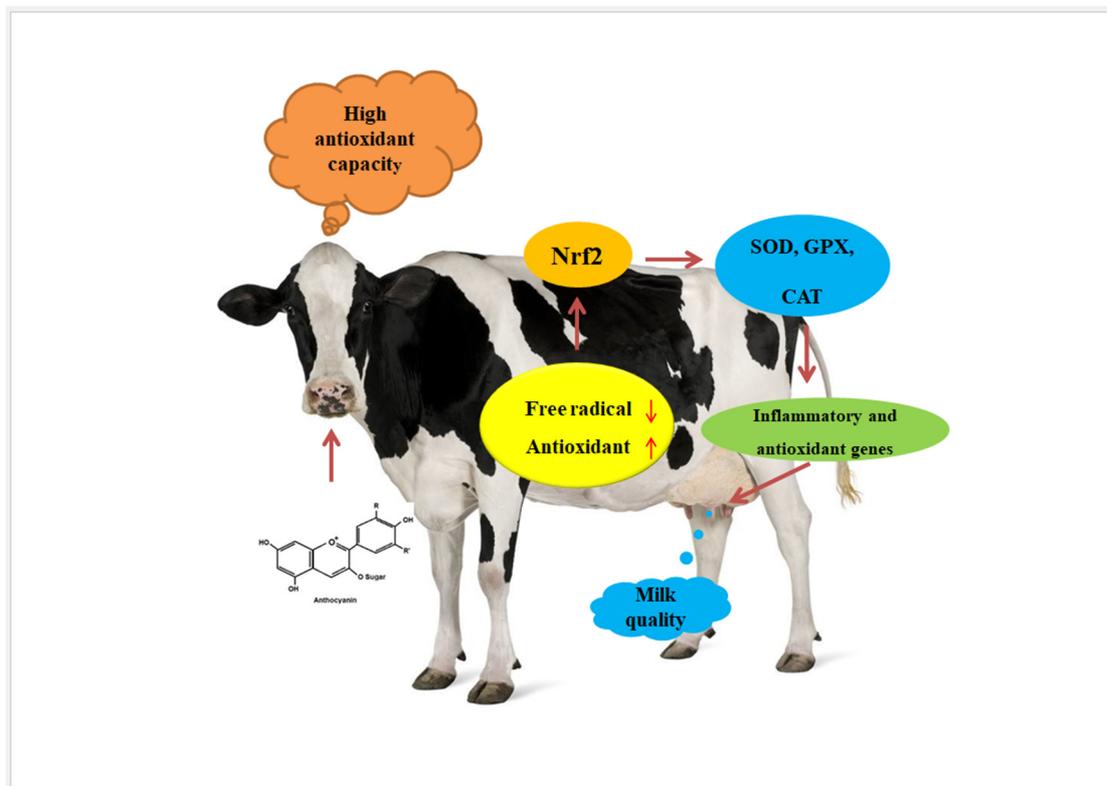


Figure 3. The postulated mechanism by which anthocyanins improve health in dairy cows.

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