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## FEEDS STUFFS WITH INCREASED ANTHOCYANIN CONTENT IN ANIMAL NUTRITION

### KRMIVA S POVEĆANIM SADRŽAJEM ANTOCIJANINA U HRANIDBI ŽIVOTINJA

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#### SUMMARY

The word anthocyanin comes from the Greek words *Anthos*, meaning flower, and *kyanose*, meaning blue. Anthocyanins give red, purple, and blue colors to plants. In acidic conditions, they appear as red pigments and in alkaline conditions, they appear as blue pigments. The intensive nature of agricultural (animal) production, with a high density of individuals in animal production facilities, very often contributes to the occurrence of disorders and diseases in animals that threaten this production. A major problem for the animal organism is the effects and consequences of oxidative stress or excessive production of free radicals, which are the main cause of metabolic disorders in cows in the transition period, and antioxidants can prevent or at least mitigate these conditions. Anthocyanins as a source of natural antioxidants play an important role in increasing the antioxidant potential that protects the cell against oxidative damage. Some anthocyanin species may have an enhancing effect on mRNA expression and superoxide dismutase activity. The bioavailability of anthocyanins is relatively low due to poor absorption in the digestive tract. In our region, colored corn, wheat, barley, and sorghum can be used as anthocyanin sources in the domestic animal diet, black soybean, rye, and black and red rice also have high anthocyanin content. Purple corn can accumulate much more anthocyanins than commercial corn varieties.

Keywords: anthocyanins, animals, feeds stuff, antioxidative effect, biological effect

#### INTRODUCTION

The ubiquitous busy lifestyle and the world population of over 7 billion people are increasingly contributing to the intensive production of food for the human population and feed for the animal population. Such an intensive way of agricultural production, with a high density of individuals in production facilities for animals, very often contributes to the occurrence of disorders and diseases in animals

that threaten this production. Various feed additives, such as antibiotics, have a preventive effect to maintain animal health and productivity. However, in the last few decades, there have been controversial, conflicting views on the consequences of consuming meat and meat products, milk, and dairy products from animals raised in this manner on the human body's well-being. One of the many consequences is the resistance of pathogenic bacteria

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to antibiotics (Gheishar and Kim, 2017). A major problem for the animal organism is the effects and consequences of oxidative stress, or the excessive production of free radicals, which is the main cause of metabolic disorders in cows during the transition period. Antioxidants can prevent or at least mitigate these conditions (Castillo et al., 2013; Tian et al., 2022). In order to solve this problem, scientists are conducting various research that will make it possible to prevent pathological conditions and disorders in animals and, consequently, the harmful effects of meat and dairy products from these animals on the final consumers. Considerable research is being conducted on developing phyto-genic additives in animal feed, triggered by the ban of antimicrobial agents as growth promoters under European Union regulations in 2006 (Karaskova et al., 2015). Since then, many food additives, such as flavonoids, which include anthocyanins, have been recommended as an alternative to antibiotic therapy (Kalantar, 2018). Studies by Suman et al. (2015) and Stold et al. (2016) have confirmed that flavonoid compounds (including anthocyanins) can reduce oxidative stress in ruminants. Considering the above, this manuscript aims to investigate and consolidate knowledge on the benefits of anthocyanins in animal nutrition, encourage critical thinking, and reach a conclusion that could serve as an answer to the question of whether the use of anthocyanins from natural sources can serve as an alternative to the use of certain bioactive components (antibiotics) that are regularly used as additives in the feeding of domestic animals and may have potentially harmful effects on the organism of the end users (humans). Considering the modest knowledge about anthocyanins, this research aims to describe anthocyanins, their properties, anthocyanin absorption, metabolism, and excretion. It also aims to describe the beneficial effects of anthocyanins on the breeding of certain domestic animals, as well as on other animals and feeds that can be used as a good anthocyanin source.

## ANTHOCYANINS

Anthocyanin comes from the Greek words “*anthos*”, meaning flower, and “*kyanose*”, meaning blue-purple color. After chlorophyll, anthocyanins are the most important group of pigments visible to the human eye. Anthocyanins belong to a wide-spread

group of water-soluble pigments, the flavonoids, which make up the majority of polyphenols. They are found in most higher plants, where they are responsible for the color of flowers, fruits, leaves, and tubers. However, anthocyanins do not appear to be present in liverworts, algae, and other lower plants, although some have been found in mosses and ferns (Delgado-Vargas et al., 2000). Anthocyanins are responsible for the red, purple, and blue colors of plants (flowers, fruits, leaves, tubers) (Escrignano-Bailón et al., 2004). Under acidic conditions, it appears as a red pigment, and under alkaline conditions as a blue pigment. Anthocyanins are vacuolar pigments found in flowers mostly in the epidermal cells and only occasionally in the mesophyll, whereas in the leaves of rye (*Secale cereale*), they are restricted to the mesophyll cells. In general, the anthocyanin concentration in most fruits and vegetables varies between 0.1 and 1% (Swain and Bate-Smith, 1962). Anthocyanin is a flavonol derivative with a basic structure of flavylium cations and is ionic. Red-colored anthocyanin pigments are predominantly in the form of flavylium cations, so they are more stable at lower solution pH values and show a variety of colors in the pH range of 1-14 (Von Elbe and Schwartz, 1996; Khoo et al., 2017). Anthocyanins are glycosides, polyhydroxy and polymethoxy derivatives of 2-phenylbezopyrylium or flavylium salts, which are broken down into anthocyanidins and sugars by hydrolysis (Amić, 2008). Anthocyanins are in the form of glycosides. The common plant pigments analogous to anthocyanins are also sugar-free anthocyanidins. Anthocyanidins found in plants are classified according to the number and position of hydroxyl groups on the flavan core. The main types of anthocyanidins are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin. By connecting anthocyanidins with glycosidic bonds, anthocyanins are formed. In nature, instead of typical anthocyanins, we also find acylated anthocyanins (Khoo et al., 2017). The stability of anthocyanins depends on the chemical structure of anthocyanins, solution pH, temperature, presence of oxygen, light, metal ions, enzymes, and other antioxidants (ascorbic acid) (Khoo et al., 2017). According to research by Giusti et al. (1999), differences in chemical structure have a major influence on anthocyanin color. The rate of degradation of anthocyanins increases with increasing temperature

(Maccarone et al., 1985). The effect of oxygen on the stability of anthocyanins is shown by the reaction of anthocyanins with peroxy radicals, with anthocyanins acting as antioxidants, which is considered the main feature of the effect of anthocyanins in the prevention of cardiovascular disease. Exposure to light has a very large effect on most flavonoids, as light is necessary for the biosynthesis of anthocyanins, but accelerates their degradation (Ćujić, 2013). Glycosidase enzymes cleave the covalent bond between the glycosyl units and the aglycone, resulting in a very unstable anthocyanidin (Huang, 1955). The stability of acylated anthocyanins increases in the presence of ascorbic acid (Poei-Langston and Wrolstad, 1981). Copigments are electron-rich compounds that bind to anthocyanins. Copigments bind with flavylium ions and have an effect on the leaf, flower and fruit colour (Trouillas et al., 2016). Ahmadiani et al. (2012) reported that heat treatment at a maximum of 35 °C reduced the total anthocyanin content in common grapes to less than half the amount in control barriers at 25 °C. At temperatures up to 40 °C the color of anthocyanins changed from red to orange, although the pH of the solution was low. Heat treatment of an anthocyanin-rich solution extract may not result in the degradation of the anthocyanin pigment because the extract normally contains phenolic compounds that are degraded by the enzyme polyphenol oxidase. Mild heat treatment of the extract at up to 50 °C has been found to inactivate the enzyme reaction (Patras et al., 2010). Therefore, mild heat treatment of raw foods such as blanching can prevent the oxidation of anthocyanins by enzymes.

#### *Function of Anthocyanins*

Anthocyanins have a dual function as active biocomponents. The first is technological, that is, its effects on the sensory properties of foods, and the second is biological, that is, its effects on health, of which the cardioprotective effect is the most important (De Pascual and Sanchez, 2008). Anthocyanins, as one of the bioactive components of functional foods, have been traditionally used as part of herbal medicine to stimulate appetite, stimulate the liver to secrete bile, and treat many other diseases (Khoo et al., 2017). Numerous studies have proven that polyphenols have various properties that have a positive effect on the human and

animal body. Some of these properties include antioxidant, anti-inflammatory, antiviral, antimicrobial, antiproliferative, antimutagenic, anti-obesity, and anticarcinogenic properties (Ghosh and Konishi, 2007), as well as antiallergic properties and prevention of osteoporosis (Fernandes et al., 2018). The same authors note that polyphenols play a role in preventing peripheral capillary fragility and preventing diabetes, as well as improving and maintaining vision. According to Allen and Tresini (2000), reactive oxygen species (ROS) cause physiological stress and shift the oxidative balance, which can lead to degenerative changes such as cancer, aging, and metabolic diseases. Anthocyanins have a stronger antioxidant effect than  $\alpha$ -tocopherol (Wang et al., 1997; Fukumoto and Mazza, 2000), trolox, and catechin (Kähkönen and Heinonen, 2003). Anthocyanins are very strong antioxidants *in vitro*, but it is not possible to say with certainty whether a diet rich in anthocyanins significantly increases antioxidant activity *in vivo*, in the body (Walton et al., 2006), especially since some other factors may influence their antioxidant activity in the host body (Pojer et al., 2013, Faehrich et al., 2015). Oxygen radical absorbance capacity (ORAC) analysis (Wang et al., 1997) is used to evaluate the antioxidant potential of anthocyanins and their glycosylated derivatives and to determine whether they possess the antioxidant activity of vitamin E (Tsuda et al., 1996). According to Tian et al. (2019), anthocyanins, as a source of natural antioxidants, play an important role in increasing the antioxidant potential that protects the cell from oxidative damage. A mixture of plant flavonoids rich in anthocyanins shows a potential effect on the protection of DNA (deoxyribonucleic acid) from damage, enzyme inhibition, estrogen activity (changing the development of diseases resulting from hormone activity), strengthening immunity (triggering cytokinin production), lipid peroxidation, reducing capillary permeability and fragility and robustness of membranes (Lefevre et al., 2004). Anthocyanins extracted from plants are used as additives in the human and animal food industry. Polyphenols, including anthocyanins, have excellent *in vitro* antioxidant activity, which depends on the polyphenol structure (Cai et al., 2006). Anthocyanins reduce the occurrence of peroxisomes and lysosomes, which involves the metabolism of  $H_2O_2$ . Research has found that the antioxidant activity of cereals can be

influenced by the genotype and maturity of the cereal (Harakotr et al., 2014). Recently, acylated anthocyanins (phenolic acid) have been increasingly used as additives in the food industry due to their higher stability and stronger antioxidant activity compared to non-acylated anthocyanins (Khoo et al., 2017). Inflammatory processes are related to the activation of cyclooxygenase (COX) enzyme activity that converts arachidonic acid to pro-inflammatory cytokines (prostaglandins). According to Seeram et al. (2001) anthocyanins significantly decreased cyclooxygenase (COX) enzyme activity. Tsoyi et al. (2008) reported that anthocyanins induce anti-inflammatory effects via downregulation of stress-activated protein kinase signaling pathways. The antimicrobial effect of anthocyanins is based on their ability to destroy the membrane, which leads to the condensation of cellular material (Cisowska et al., 2011). In addition, anthocyanins can destroy substrate microbes by inhibiting extracellular enzymes (Naz et al., 2007) that directly affect microbial metabolism (Burdulis et al., 2009). In addition, anthocyanins can positively influence the growth of beneficial bacteria such as *Bifidobacterium spp.*, *Lactobacillus spp.*, and *Enterococcus spp.* (Pojer et al., 2013). Anthocyanins also have many other therapeutic effects, such as curing *Staphylococcus aureus* infections as a source of antiphlogistic or immunosuppressive active ingredients (Roewer and Broscheit 2013). The anthocyanins delphinidin and delphinidin-3-glucoside have inhibitory effects on hydroxyl radical scavenging activity, lipid peroxidation, O<sub>2</sub> scavenging activity, and low-density lipoprotein (LDL) oxidation. Several studies have found that anthocyanins have an antiangiogenic effect on the prevalence of various human diseases, including cancer and diabetic complications such as diabetic retinopathy and nephropathy (Xue et al., 2010). Anthocyanins show antithrombotic effects *in vitro* by improving lipid profile and platelet function (Alvarez-Suarez et al., 2014). Anthocyanidin and anthocyanin pigments have anti obesity properties (Tsuda et al., 2003). In the study, the authors demonstrated that hyperglycemia, hyperinsulinemia, hyperleptinemia, and elevated tumor necrosis factor (TNF- $\alpha$ ) mRNA levels that occurred in obese rats were normalized after nutrition with a purple corn diet rich in anthocyanins.

### *Absorption, metabolism, and excretion of anthocyanins*

After consuming a diet rich in anthocyanins, the bioactive ingredients still have a long way to go before they have a property that benefits the health of the consumer. They must pass through the oral cavity and gastrointestinal tract, undergo metabolism, pass through the cell barrier, and finally trigger specific activity (Fernandes et al., 2018). Anthocyanins are large, water-soluble molecules that were thought to be difficult to absorb into the cells or bloodstream of animals and humans. Recent absorption studies show that anthocyanins are absorbed from the gastrointestinal tract, transported into the bloodstream, and then excreted in the urine. Although there are numerous studies investigating the absorption and bioavailability of anthocyanins compared to other flavonoids, relatively little is known about the absorption and transport mechanisms of anthocyanins (McGhie and Walton, 2007). Several studies have shown that absorption of anthocyanins may begin in the stomach, with anthocyanins appearing in the blood as early as 6 to 20 minutes after biting, and the highest anthocyanin content in the blood was found after 15 to 60 minutes (Changxing et al., 2018). The bioavailability of anthocyanins is relatively low because absorption in the digestive system is weak and depends on the type of compound, chemical structure, extent of conjugation, and individuality of intestinal microflora (Fumić, 2016). Intestine micropopulation plays an important role in anthocyanin bioavailability (Fernandes et al., 2018). Other factors on which the absorption of anthocyanins in the body depends include the nature and chemical structure of the food, the degree of food processing and preparation, the interaction with other phytonutrients, and also pathophysiological, nutritional, and genetic characteristics of the individual (Riaz et al., 2016). The nature and chemical composition of food, the degree of food processing and preparation, interactions with other phytonutrients, and pathophysiological, nutritional, and genetic characteristics of individuals are additional variables that affect anthocyanin absorption in the body (Riaz et al., 2016). Several studies have shown that the small intestine is the most important site for anthocyanin absorption (Changxing et al., 2018). Absorption of anthocyanins generally occurs

through an active transport mechanism, as these relatively large molecules would otherwise need to be hydrolyzed into  $\beta$ -glycosides before absorption (Riaz et al., 2016). The mechanism of active transport refers to the way of transporting whole molecules through the cell membrane with the help of membrane enzymes. Another possible transport mechanism is the transfer of intact glucosides via the sodium-glucose co-transporter or extracellular glycoside hydrolysis, followed by passive diffusion of the final product. It should also be noted that anthocyanins cannot be absorbed by passive diffusion due to their hydrophilic property and molecular size (Changxing et al., 2018). After anthocyanins are absorbed through the mucosa of the gastrointestinal tract, they are converted to methylates, sulfates, and glucuronides by enzymes in the kidneys and liver (Changxing et al., 2018). These forms may enter the jejunum with bile and be returned to the small or large intestine via enterohepatic circulation. Some of the anthocyanins may re-enter the jejunum via bile, be absorbed through the colon and enter the enterohepatic circulation, or be excreted with feces. Excretion of anthocyanins occurs mainly through the urine (McGhie et al., 2003). A review of the literature reveals contradictory research findings. Walle (2004) believes that the lungs are the main excretory organ for many flavonoids, but Changxing et al. (2018) note that there is no claim in the literature to suggest that anthocyanins are excreted from the body through respiration. It has been shown that the bioavailability of anthocyanins is very low, so the amount absorbed and excreted in the urine may be less than 0.1% of the anthocyanin dose taken. According to Tian and Lu (2022), the uptake and metabolism of anthocyanins in ruminants are still unclear. Anthocyanin-rich plants used in animal feeding as feedstuff also have other natural antioxidants such as flavonoids, polyphenols, and vitamins not only anthocyanins. Therefore, further *in vivo* studies in ruminants are needed to determine the degradation pathways of absorption and excretion of individual anthocyanin components.

#### *Use of anthocyanins in animal feeding*

Intensive agricultural production often encounters numerous problems during the production cycle. Various diseases and disorders occur as a result of poor environmental conditions, heat stress, the occurrence of viruses, bacteria and other pathological conditions. Therefore, it is necessary

to prevent such disorders and pathological conditions, the consequences of which can be significant economic losses.

#### *Use of anthocyanins in the feeding of non-ruminants*

Anthocyanins have shown preventive and therapeutic effects against various and numerous diseases (Changxing et al., 2018). The addition of safflower seed cake (*Silibum marianum* L.) containing 129.83 mg/kg cyanidin-3-glucoside to the feed for broilers significantly improved the tenderness, color, flavor, and other sensory properties of the leg drumstick and breast meat of these chickens (Šťastník et al., 2016a). The addition of purple corn to the rations of laying Japanese quail improves eggshell quality, laying performance, and carcass quality (Amnueysit et al., 2010). Anthocyanins have a positive effect on heat stress. In the study by Hayati et al. (2015), who added grape seed extract to broiler meals, a significant contribution to improving growth performance was found. In addition, the aforementioned authors found that the deleterious effects of heat stress in birds were suppressed by the fact that grape seed extract lowered blood glucose and cholesterol levels, as well as heat shock protein (HSP70) gene expression. In an *in vivo* experiment, Pashtetsky et al. (2019) found that grape seed extract inhibited lipid oxidation in poultry during gastric digestion. In a study conducted on rats fed meals composed of blue wheat with a cyanidin-3-glucoside content of 47.63 mg/kg, Šťastník et al. (2016b) found a significant decrease in plasma cholesterol in experimental rats compared with control rats. Also, Šťastník et al. (2016a) found in another study that the addition of purple wheat (cyanidin-3-glucoside 36.66 mg/kg) at the level of 60% of the ration showed no positive effect, but also no negative effect in broilers. In the study of Amnueysit et al (2010), when purple corn was added to broiler rations at 30% and 40%, lower heart weight of broilers was found, 0.48 g and 0.44 g, compared to the control group, 0.55 g. The same authors note that no significant differences in heart mass were found in groups of broilers with 20% and 60% added purple corn in the rations (0.52 and 0.53, respectively). In addition, the authors found a significant reduction in abdominal fat in broilers fed purple corn compared to the group fed only yellow corn. Research results indicate that purple corn has the potential to reduce the percentage of heart mass relative to carcass mass, as well as the

degree to which carcasses are fattened with abdominal fat. The authors did not establish connection between purple corn and carcass quality. In the study conducted by Mrkvicova et al. (2016) with broilers fed only wheat with different anthocyanin content, higher consumption but lower individual growth was observed after 15 days in the group fed purple wheat compared to the control group fed normal wheat. Surai, (2014) finds that proanthocyanidins from bean extract significantly inhibit digestive enzymes such as trypsin,  $\alpha$ -amylase, and lipase in young chickens. Table 1 shows the features of the action of polyphenols in poultry nutrition.

Hosoda et al. (2012a) reported that anthocyanins increase superoxide dismutase (SOD) activity in monogastric animals. Superoxide dismutase is an important antioxidant enzyme whose function is to prevent the occurrence of oxidative stress (Hosoda et al., 2012a). The same author states that the consumption of a meal rich in anthocyanins in non-ruminants leads to an increase in the total antioxidant capacity of the organism. Daily flavonoid supplementation increases the ability of birds to primary immune response to novel antigens (Faehrich et al., 2016).

#### *Use of anthocyanins in feeding of ruminant*

Numerous studies indicate that bioactive compounds from the flavonoid group can influence the production of volatile fatty acids and also reduce methane concentration in the rumen, ultimately leading to improved growth and production performance and a positive environmental effect. Flavonoids can also promote the growth and development of animals and improve the quality of animal products. Therefore, these compounds are often used as feed additives instead of antibiotics (Kalantar, 2018). Researchers are very interested in investigating the potential of bioactive components capable of modifying the rumen micropopulation and inducing desirable changes in fermentation conditions, e.g., pH changes, propionate production, protein degradation, and control of nutritional stress such as flatulence and acidosis. Recently, the use of flavonoids and other polyphenolic compounds as additives in ruminant feeding has gained importance (Kalantar, 2018). Contrary to monogastric animals, ruminants have been shown to benefit from the strong antioxidant properties of polymeric proanthocyanidins by metabolizing them into bioavailable components with epicatechin as an intact flavonoid ring

**Table 1 Effect of polyphenols on the poultry organism (Surai, 2014)**

**Tablica 1. Utjecaj polifenola na organizam peradi (Surai, 2014.)**

Effect - Utjecaj	Polyphenols - Polifenoli
Structure - Struktura	Many different compounds with differences in structure Mogo različitih sastojaka različite strukture
Absorption - Apsorpcija	Poor absorption Slaba apsorpcija
Metabolic transformation in tissue Metabolička transformacija u tkivu	Rapid transformation into various metabolites Brza transformacija u različite metabolite
Delivery to the target tissue Dostava do ciljanog tkiva	The concentration in the tissue is insignificant Konzentracija u tkivu neznatna
Impact on nutrient digestion Utjecaj na probavu nutrijenata	Reduced digestion of many nutrients including proteins and lipids Smanjena probava mnogih nutrijenata uključujući bjelančevine i lipide
Effect on reproduction Utjecaj na reprodukciju	No proven effect Nema dokazanog utjecaja
Immune effects Utjecaj na imunitet	The effects on immunity are not constant Utjecaji na imunost nisu konstantni
Antioxidant properties Antioksidativna svojstva	Depending on the conditions, they can be antioxidants or pro-oxidants Ovisno o uvjetima, mogu biti antioksidansi ili pro-oksidansi
Toxicity Toksičnost	They could be harmful if consumed in large quantities Mogli bi biti štetni pri većoj konzumaciji

structure (Kalantar, 2018). The bioavailability of proanthocyanidins in ruminants is higher compared to other flavonoid classes, which is in contrast to monogastric animals, where the bioavailability of isoflavones and flavonols is higher than that of anthocyanins (Kalantar, 2018). Tian et al (2019) consider that small ruminants such as goats are more susceptible to oxidative stress due to their intensive metabolic demands in husbandry and production. The occurrence of oxidative stress accelerates the occurrence of diseases that lead to the occurrence of mastitis and reproductive disorders, but also possible infections with parasites, and as a final result, there is a decrease in milk production. Anthocyanins have a positive effect on the antioxidant defense system of ruminants. The beneficial effects of flavonoids and phenolic compounds on animal production and health, rumen fermentation, reduction of methane production, and prevention of feeding stress such as rumen bloat or acidosis have been proven in several cases (Kalantar, 2018). Hosoda et al. (2012b) conducted a study in which anthocyanins from purple corn (*Zea mays L.*) were fed to sheep to investigate their effects. Sheep were divided into two equal groups, and one group was fed a meal containing purple corn, while the control group was fed regular corn. In urine and blood samples and by measuring oxidative status parameters, it was found that the purple corn-fed group had a significant increase in plasma superoxide dismutase activity, although the total antioxidant content and plasma glutathione concentration in both groups were the same when oxidative resistance was tested. They also found that the oxidation level in the blood plasma of the purple corn-fed group was significantly lower compared to the control group. Numerous studies conducted on small ruminants such as goats and sheep have shown that oxidative stress can be induced by various factors such as feeding a large amount of fat, physiological maturity, season, and pregnancy (Hosoda et al., 2012b). Some types of anthocyanins may have an enhancing effect on mRNA expression and the activity of superoxide dismutase, which is an important antioxidant enzyme in living organisms (Hosoda et al., 2012b). High-producing dairy cows are exposed to oxidative stress caused by active oxygen, and this stress is more pronounced at increased milk production and under heat stress conditions (Matsuba et al., 2019). Active oxygen is necessary to protect animals from pathogens, but it also

damages animal tissues if not properly removed; it is necessary to include antioxidants in the feeding of dairy animals. Anthocyanin, a plant polyphenol, has antioxidant activity both *in vitro* (Tsuda et al., 1994; 1996; Gabrielska et al., 1999) and *in vivo* (Seeram et al., 2006; Cimino et al., 2007). Total antioxidant status in dairy cows decreases during lactation (Castillo et al., 2006). According to Sies, (1997), oxidative stress occurs when the production of free radicals suppresses the capacity of the organism's antioxidant system. Matsuba et al. (2019) observed that dairy cows fed purple corn silage had higher concentrations of superoxide dismutase and had higher milk production than the control group fed conventional corn silage. Prommachart et al. (2021) found that black rice and purple corn extracted residue supplementation up to 6% in the diet of male dairy cattle had no effect on feed intake, nutrient digestibility, and growth performance. However, cattle fed with extracted residue of black rice and purple corn had significantly ( $P < 0.05$ ) lower concentrations of malondialdehyde in plasma, indicating lower lipid peroxidation. Anthocyanins from red cabbage extract increased the ruminal concentration of total volatile fatty acids and the molar population of propionate in the rumen and decreased the acetate to propionate ratio in beef bulls (Gao et al., 2022). In addition, the authors found that anthocyanins from red cabbage extract increased the relative abundance of *Ruminobacter*, *Anaerovibro*, *Oribacterium*, and *Monoglobus* and tended to increase plasma concentrations of globulin and total protein, but had no effect on antioxidant indices in beef bulls. The authors concluded that anthocyanins from red cabbage extract are highly hydrolyzable in the rumen fluid and must be encapsulated in the rumen to protect them from degradation. Tian et al. (2019) observed increased levels of lactose in the milk of a group of goats fed purple corn silage compared to a group of goats fed regular corn silage. In the aforementioned study, the authors assume that this increase is due to the effect of purple corn silage on fermentation in the rumen, especially the inhibition of acetic acid and the increase in the proportion of propionic acid in the rumen. They also believe that the sugars contained in anthocyanins can be broken down in the digestive tract and thus participate in the synthesis of lactose. To understand the stability of anthocyanins during silage preparation, the relationship between the stability of anthocyanins and lactic acid



fermentation during ensiling should be established, since anthocyanins consist of anthocyanidin and sugar, there is a possibility that anthocyanin sugar can be used as a substrate for lactic fermentation. In order to offer ruminants silage with an accurately known amount of anthocyanins, it is important to understand the movement of quantitative changes in anthocyanin levels during storage (Hosoda et al., 2009). Incubation of purple corn with rumen fluid did not result in the degradation of anthocyanins, leading the authors to conclude that the anthocyanin in purple corn is protected from digestion in the rumen and as such can be absorbed by ruminants (Hosoda et al., 2009). According to research, the anthocyanin in anthocyanin-rich corn appears to be protected from digestion by ruminants and therefore can be absorbed by ruminants. In addition, anthocyanin-rich corn appears to be suitable for providing antioxidant substances to dairy cattle due to the stability of its anthocyanins in the rumen fluid. However, the potential effect of anthocyanin-rich corn needs to be verified in an *in vivo* experiment in ruminants. Tian et al. (2019) recommend the use of purple corn silage as a bulking agent in ruminant diets for 3 reasons:

- it has no harmful effect on the components of milk
- improves oxidative resistance
- the composition of anthocyanins can be transferred to milk in goats.

Tian et al. (2021) found in goats receiving purple corn pigment had increased ( $P < 0.05$ ) ruminal fluid acetic acids and a higher ratio of acetate to propionate, while the propionic acid, butyric acid, valeric acid, isobutyric acid, and isovaleric acid had decreased ( $P < 0.05$ ). In addition, the authors found significantly increased ( $P < 0.05$ ) levels of reduced glutathione and peroxidase in the blood plasma of goats that received purple corn pigment compared with control goats. Anthocyanins from purple corn affect rumen microflora by regulating the relative abundance of rumen microbes and improving rumen microbial diversity. Antunović et al. (2022) found no effect of red corn in a feed mixture for lambs on their productive characteristics or most hematological and biochemical blood indicators. However, significantly ( $P < 0.05$ ) higher blood hemoglobin content and increases in serum aspartate aminotransferase and creatinine kinase activity were found in

a group of lambs where yellow corn was 100% replaced with red corn, as well as decreased serum glucose and non-esterified fatty acids compared to a group of lambs fed yellow (regular) corn. Purba et al. (2022) determined that feeding a goat with a total mixed ration with 50% treated anthocyanin-rich black cane treated with 0.030% ferrous sulfate heptahydrate reduces oxidative stress and makes the meat more tender, with no effect on dry matter intake or growth performance. Maggiolino et al. (2021) studied the effects of dietary supplementation of suckling lambs with anthocyanins from red-orange and lemon extracts on growth performance, carcass, oxidative and meat quality traits. During the 40-day experimental period, anthocyanins had no effect on live weight at slaughter (12.2 and 12.4 kg in experimental and control animals, respectively), carcass measures, dressing percentage (64.2% and 65.1% in experimental and control animals, respectively). Lambs meat supplemented with red-orange and lemon extract had significantly lower ( $P < 0.05$ ) cooking loss and ( $P < 0.01$ ) Warner-Blatzer Shear Force values. Thiobarbituric acid reactive substances and hydroperoxides were also lower in meat anthocyanin supplemented lambs. Moreover, red-orange and lemon extracts affect yellowness values showing higher ( $P < 0.01$ ) values in supplemented lambs. Superoxide dismutase and glutathione peroxidase were higher in supplemented lamb meat ( $P < 0.01$ ), indicating increased antioxidant activity and oxidative stability. The aforementioned resulted in more attractive meat for consumers, possibly longer shelf life, and healthier meat for human consumption.

#### *Use of anthocyanins in the feeding of other animals*

Studies have shown that in horses fed a certain amount of anthocyanins, the alanine aminotransferase and creatine kinase enzymes in the muscle after work are lower, which proves the protective effect of anthocyanins on the horse's muscles (www.Ranvet.com, 2018). Increased dietary anthocyanin intake also has an effect on reducing leukocyte breakdown. Grape seed extract, which is rich in anthocyanins and proanthocyanins, may play an important role in preventing acidosis, which can lead to colic in horses (Oke, 2020). Mrkvicova et al. (2016) studied the influence of the addition of purple wheat with a higher content of anthocyanins on antioxidant activity and liver function in different

animal species (rats, chickens, and fish). The experimental animals were fed Purple Konini wheat with a total anthocyanin content of 41.70 mg/kg and the control animal common wheat with a total anthocyanin content of 24.95 mg/kg. The feeding experiment lasted 23 days in rats, 15 days in chickens, and 34 days in fish. The authors found that feeding purple Konini wheat with higher levels of anthocyanins had no effect on the feed intake or production characteristics of the animals. In contrast, significantly ( $P < 0.05$ ) higher values of antioxidant status, as measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl test), FR (Free Radical) method, were determined in the liver of rats fed purple wheat. Chickens fed purple wheat showed significantly higher values by DPPH and ABTS (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) test and lower values measured by the method FR. Significantly ( $P < 0.05$ ) lower gamma-glutamyltransferase (GMT) activity was found in chickens fed purple wheat. In general, the authors concluded that purple Konini wheat can improve the antioxidant activity and function of liver tissue. Stasnik et al. (2019) observed lower average feed intake and carcass weight in a study with rabbits fed a 15% purple wheat feed mixture. Matsu-moto et al. (2006) introduced black currant anthocyanins intravenously into the bodies of rabbits, demonstrating that all four different anthocyanins found in black currant reach the eye tissue intact. Hsieh et al. (2016) find that antioxidants, such as phenolic compounds and anthocyanins, in black soybean seed coats may have a beneficial effect on fish growth.

#### FEEDS WITH INCREASED ANTHOCYANIN CONTENT

In addition to the nutritional value of some feeds and antinutritional substances, researchers are currently investigating biologically active substances with protective and preventive properties against degenerative diseases and other health problems to extend their lifespan (Ficco et al., 2014). In addition to grain genetic characteristics, physiological and environmental growth conditions, extraction, identification, and quantification methods can greatly influence the results of qualitative and quantitative tests of anthocyanins in various plant development studies (Zhu, 2018). Recently, colored cereal grains have attracted much attention because of their

attractive nutritional value. The main type of pigment responsible for the colors, as well as the health benefits, of grains is anthocyanins (Zhu, 2018). The main cereals of global importance with elevated anthocyanin content are corn (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum spp.*), barley (*Hordeum vulgare*), and sorghum (*Sorghum bicolor*). Cereals of local importance with increased anthocyanin content are millet (*Panicum miliaceum*), oats (*Avena sativa*), rye (*Secale cereale*), and some others.

#### Colored corn

This variety of corn has the ability to accumulate much more anthocyanins than commercial corn varieties, which is why it is considered a good source of antioxidants in animal nutrition (Hosoda et al., 2009). There is a great genetic diversity in the color of corn kernels, so there are black, blue, pink, red, and even brown corn kernels (Zhu, 2018). In recent decades, the functional properties of purple corn have become of increasing interest to scientists thus becoming more the subject of research (Lao et al., 2017). A corn genotype with anthocyanin-rich endosperm has not yet been developed, but corn ranks first in anthocyanin content (Zhu, 2018). Purple corn kernels have higher anthocyanin content [891-3,312 CGE (cyanidin-3-glucoside equivalent)  $\mu\text{g/g}$ , db (dry basis)] than blue (up to 540 CGE  $\mu\text{g/g}$ , db) and red (up to 127 CGE  $\mu\text{g/g}$ , db) (Collison et al., 2015). Hosoda et al. (2009) studied the fermentation quality and quantitative changes of anthocyanins in purple and ordinary corn during storage and in vitro rumen fermentation. A significantly higher lactic acid concentration was found in purple corn silage compared to normal corn silage on all days after ensiling. It has been shown that purple corn silage (in silos - barrels) has significantly lower pH and ammonia concentration after 60 days of storage, while lactic acid concentration is significantly higher compared to ordinary corn silage. The amount of anthocyanins during ensiling, that is, on the zero day of storage, in the experiment was 3.34 mg/g of dry matter of purple corn silage. The amount decreased significantly until the 60th day, after which the content in the silage remained constant until the 180th day of storage (1.88 mg/g of dry matter), which corresponds to about 45% of the initially measured amount of anthocyanins. Purple corn is a maize native to Peru. Given the richness of the purple color, it has long been used as a colorant

for food and beverages. In the last decade, the use of purple corn as an additive to food and beverages has increased significantly, as evidenced by data on the importation of purple corn into European Union countries such as Germany, France, and Italy, as well as other countries in the world such as Japan (Lao et al., 2017). Because of its extraordinary impact on consumer health, several commercial companies proposed in 2013 that purple corn be granted “superfood” status (Lao et al., 2017). In terms of chemical composition and nutritional values, purple corn is similar to commonly used regular corn in terms of its high content of starch (61% to 78% of dry matter), non-starch polysaccharides (about 10% of dry matter), protein (6% to 12% of dry matter), fat (3% to 6% of dry matter), minerals, and vitamins (Ai and Jane, 2016). The composition of anthocyanins in purple corn has been well studied, and 6 more important and 17 less important anthocyanins have been identified (Lao et al., 2017). Phenols from purple corn showed higher antioxidant capacity and faster kinetic response compared to the same amount of phenols from blueberries, proving that phenols from purple corn have a greater number of active hydroxyl groups and a more favorable configuration for better interaction with free radicals (Cevallos-Casals and Cisneros-Zevallos, 2003). The antioxidant capacity of purple corn remained at a high level after industrial processing (Del Pozo - Insfran et al., 2006). More than 20 bioactive phenolic components such as phenolic acids, anthocyanins, and other flavonoids have been found in purple corn (Lao et al. 2017). Properties that can contribute to consumer health have been demonstrated in many studies. Lao et al (2017) list some of them: anticancer properties, antimutagenic properties, antioxidant properties, anti-inflammatory properties, antidiabetic properties, regulation of blood pressure and heart rate, and prevention of obesity.

#### *Colored wheat*

Colored wheat, which is rich in anthocyanins, is of interest to scientists and the food industry because of its potential use as a food colorant, nutrient, and functional food (Manach et al., 2003). Common wheat cultivars that we find are usually white. Colored wheat is rather rare to find. The purple color is found in the pericardium of the grain, while the blue color is found in the aleurone of the grain (Abdel-

Aal et al., 2008). There is interest in growing colored wheat, but its main problem is low yield (Sharma et al., 2018). Total anthocyanin content is lowest in white wheat, higher in colored wheat varieties, and significantly highest in black wheat (Sharma et al., 2018). The antioxidant activity of white wheat is mainly associated with its phenolic acids, while in colored wheat the high anthocyanin content has an additional effect on antioxidant activity (Sharma et al., 2018). Although purple wheat has a lower total anthocyanin content compared to blue and black wheat, it has been shown to have a stronger inhibitory effect on the production of anti-inflammatory cytokines, which may be due to the content of larger amounts of acylated anthocyanins with better stability and bioavailability (Abdel-Aal et al., 2006). Mrkvicova et al. (2016) found that feeding wheat with increased anthocyanin content had no negative or positive effects on food consumption or the growth of animals.

#### *Barley*

Barley exhibits wide genetic variability in color (red, blue, purple) and anthocyanin content. The average anthocyanin content in seven groups of barley (127 genotypes in total) that differ in color is 60-350  $\mu\text{g/g}$  (Kim et al., 2007). In studies, Lee et al. (2013) found as many as 1.66 g/kg anthocyanins in the bran of one barley genotype. Numerous studies have determined various anthocyanin compounds in barley kernels, such as: delphinidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-malonylglucoside, cyanidin 3-malonylglucoside, cyanidin 3-(6''-succinyl) glucoside, and peonidin 3-(6''-succinyl) glucoside (Diczházi and Kursinszki, 2014; Lee et al., 2013).

#### *Sorghum*

As with barley, sorghum has been found to have wide variability in kernel color (Rhodes et al., 2014) and anthocyanin content. The aforementioned authors analyzed 381 sorghum genotypes and found wide variation in anthocyanin content (0-149 absorbance/mL/g, arbitrary unit). Dykes et al. (2009) analyzed 13 sorghum genotypes and determined the content of 3-deoxyanthocyanin up to 680  $\mu\text{g/g}$ . In addition to the kernel, i.e. the grain, the colored leaves of sorghum are also being investigated as a potential source of anthocyanins (Petti et al., 2014).

Kayode et al. (2012) analyzed the anthocyanin content in the leaf sheath of 6 color sorghum genotypes and found values ranging from 14 to 35 cyanidin-3-glucoside equivalent (CGE) mg/g. Therefore, leaf material which is usually waste can be used for anthocyanin extraction and reuse. Great genetic diversity in the type of anthocyanins was found in sorghum. For example, in sorghum kernels (13 genotypes) were determined luteolinidin (0-282  $\mu\text{g/g}$ ), apigeninidin (0-166  $\mu\text{g/g}$ ), 5-metoksiluteolinidin (0-154  $\mu\text{g/g}$ ), and 7-metoksiapigeninidin (0-137  $\mu\text{g/g}$ ). In addition, apigeninidin (17-46 mg/g), luteolinidin (0.4-2.4 mg/g), and malvidin (0.6-1.0 mg/g) were found in sorghum leaves (Kayodé et al., 2012).

#### Millet

Millet includes a range of small-seeded grains such as pearl millet (*Pennisetum glaucum*), proso (*Panicum miliaceum*), foxtail (*Setaria italica*), and finger millet (*Eleusine coracana*) (Zhu, 2018). In a study by Siwela et al. (2010), anthocyanin components in ethyl acetate extract were analyzed and the presence of apigeninidin and luteolinidin type anthocyanins was detected. The anthocyanin profile in different variegated grains needs to be investigated in the future.

#### Rye

Dedio et al. (1972) found cyanidin-3-glucoside and peonidin-3-glucoside in the coleoptile of ray and in acylated forms in the pericarp, cyanidin-3-rutinoside in the coleoptile and cyanidin-3-glucoside in the first ray leaves. In the past, there have been few reports of colored rye that have not been reviewed and colored rye genotypes have yet to be developed by genetic means (Zhu, 2018).

#### OTHER FEEDS RICH IN ANTHOCYANINS THAT CAN BE USED IN FEEDING DOMESTIC ANIMALS

Although in our area mainly purple corn, purple wheat and rye can be used as anthocyanin sources for feeding domestic animals, black and red rice should also be mentioned. Anthocyanins are mainly concentrated in paddy rice bran (Zhu, 2018). In addition to its carbohydrate value, colored rice has a very good content of anthocyanins and proanthocyanins (Abdel-Al et al., 2006). Anthocyanin content in different colored cultivars can vary from 700

to 5000  $\mu\text{g/g}$ , and proanthocyanin content can vary from 750 to 3000  $\mu\text{g/g}$  (Hosoda et al., 2018). The problem is caused by the fact that rice is almost indigestible in the rumen unless it undergoes a milling or hulling process (Hosoda et al., 2018). Black soybeans contain a large amount of antioxidants, such as phenolic compounds and anthocyanins, in the grain envelope (Hsieh et al., 2016). Black soy has anti-inflammatory and anti-proliferative effects and significant antioxidant activity (Kim et al., 2008). Lazalde-Cruz et al. (2021) studied the effect of anthocyanins delphinidin-3-O-sambubioside (DOS) and cyanidin-3-O-sambubioside (COS) from *Hibiscus sabdariffa* L. on meat and milk quality of ruminants. *Hibiscus sabdariffa* L. is a shrub adapted to spring-summer and subtropical or tropical environments. After reviewing numerous scientific articles, the authors concluded that the inclusion of *Hibiscus sabdariffa* byproducts such as stalks, leaves, and seeds in ruminant diets could reduce economic costs and environmental problems and improve the quality of meat and milk. Antioxidants from *Hibiscus sabdariffa* could affect rumen fiber durability, fermentation patterns, and bi-hydrogenation of fatty acids and reduce methane emissions. Anthocyanins from *Hibiscus sabdariffa* increase the activity of desaturase enzymes that convert monounsaturated fatty acids to polyunsaturated or add additional unsaturated bonds to existing ones, thus reducing oxidative effects on color, odor, and flavor of dairy products and meat. All of the above effects can be beneficial for the health of human consumers.

#### CONCLUSION

Numerous studies have demonstrated that anthocyanins have various beneficial effects on the human and animal body. Some of these effects include antioxidant, anti-inflammatory, antiallergic, antiviral, antimicrobial, antiproliferative, antimutagenic, and anticarcinogenic properties. Anthocyanins have antioxidant activity both *in vitro* and *in vivo*. Although research shows significant positive health effects, the benefits of feeding anthocyanin-rich diets to non-ruminants and ruminants are significantly limited by the lower absorption of anthocyanins in the digestive system of animals and depend on the type of compound, chemical structure, extent of conjugation, and individuality of the intestinal microflora.

No adverse effects have been observed when feeding anthocyanin-rich diets to ruminants and nonruminants, and several benefits have already been reported. Benefits of such feeding in poultry include improved eggshell quality and laying performance, improved growth performance, and protection against heat stress by lowering blood glucose and cholesterol concentrations. Research shows that purple corn has the potential to reduce the percentage of heart mass relative to carcass mass and fatten the carcass with abdominal fat. Anthocyanins increase superoxide dismutase activity in monogastric animals, improving the protection of the organism. In ruminants, anthocyanins influence the production of volatile fatty acids, affect the reduction of methane concentration in the rumen, and thus can improve growth and production performance. Unlike monogastric animals, ruminants have been shown to benefit from the strong antioxidant properties of polymeric proanthocyanidins by metabolizing them into bioavailable components. The positive effects of flavonoids and phenolic compounds on animal production and health, rumen fermentation, reduction of methane production, and prevention of feeding-related stress are well known. Dairy cows fed purple corn silage have higher levels of superoxide dismutase and higher milk production, while goats have higher levels of lactose in their milk. According to research, the anthocyanin in anthocyanin-rich corn appears to be protected from digestion by ruminants and therefore can be absorbed by them. Many cereal varieties and genotypes have been studied, and most have a unique composition of anthocyanins. Purple corn has the greatest potential for use as an anthocyanin-rich feed because the nutrient yield is most efficient along with the anthocyanins in the form of grains or ensiled material. Despite its great potential, anthocyanin-rich feeds are not available in sufficient quantities for feeding domestic animals in our area. Moreover, genetic diversity in anthocyanin composition has been found to depend on growing conditions, plant physiology, and plant parts. Relatively little is known about the absorption and transport mechanisms of anthocyanins; therefore, further *in vivo* research in ruminants and non-ruminants is needed to determine the degradation pathways of absorption and excretion of individual anthocyanin components.

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## SAŽETAK

Riječ antocijanin dolazi od grčkih riječi *anthos*, što označuje cvijet te *kyanose* što označuje plavu boju. Antocijanini biljkama daju crvenu, ljubičastu i plavu boju. U kiselim uvjetima se pojavljuje kao crveni pigment, a u lužnatim kao plavi pigment. Intenzivan način poljoprivredne (stočarske) proizvodnje s velikom gustoćom jedinki unutar proizvodnih objekata za životinje vrlo često pridonosi pojavi poremećaja i bolesti životinja koje ugrožavaju tu proizvodnju. Veliki problem za životinjski organizam su učinci i posljedice oksidacijskog stresa, odnosno prekomjerne proizvodnje slobodnih radikala koji su glavni uzrok metaboličkih poremećaja kod krava u prijelaznom razdoblju, a antioksidansi mogu spriječiti ili barem ublažiti ta stanja. Antocijanini kao izvor prirodnih antioksidanata imaju važnu ulogu u povećavanju antioksidacijskog potencijala koji štiti stanicu od oksidacijske štete. Biodostupnost antocijanina je relativno niska s obzirom na relativno slabu apsorpciju u probavnom sustavu životinja. Neke vrste antocijanina mogu imati pospješujući učinak na ekspresiju mRNA i aktivnost superoksid dismutaze. Na našem području kao izvor antocijanina u hranidbi domaćih životinja ponajviše se može koristiti ljubičasti kukuruz, ljubičasta pšenica, ljubičasti ječam te ljubičasti sirak, ali valja spomenuti i crnu soju, raž te crnu i crvenu rižu koji također posjeduju značajni sadržaj antocijana. Ljubičasti kukuruz ima sposobnost nakupljanja značajno više antocijanina u odnosu na komercijalne sorte običnog kukuruza.

Ključne riječi: antocijanini, životinje, krmiva, antioksidacijski učinci, biološki učinci