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Betalains in Animal Nutrition and Health: Importance as Animal Feed Supplements: A Review

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ABSTRACT

In recent years, betalain-containing fruits and vegetables have gained increased importance in research and food industry. The extracted betalains are safe to consume and are considered as health promoting molecules. *In vitro* and *in vivo* studies highlighted the antioxidant and the biological activities of these pigments. However, the bioavailability of betalains, their ability to protect/treat oxidative stress related diseases and their health promoting properties were investigated using mainly rodents and human models. To enlarge the sphere of application of these pigments, research trials should be extended to farm animals. This paper details the bioavailabilty and the *in vivo* radical scavenging activity of these molecules and proposes their use as natural animal feed additives for future debate.

Key words: Animal health, Animal nutrition, Betanin, Bioavailability, Caryophyllales, Indicaxanthin.

Secondary metabolites, or phytochemicals, are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Phytochemicals are produced not only by plants, but also by fungi and non-pathogenic endophytic bacteria living within the plants (Strobel *et al*., 2004; Verma *et al*., 2009; Yu *et al*., 2010; Bascom-Slack *et al*., 2012; Aly *et al*., 2013; Mousa and Raizada, 2013). They serve as (i) a defence mechanism against pathogens; (ii) metal transporting agents; (iii) symbiotic agents; (iv) sexual hormones and (v) differentiation factors (Demain and Fang, 2000). The most common phytochemicals are chlorophylls, phenolic acids, flavonoids, carotenoids, anthocyans and betalains (Kumar *et al*., 2019; Garg, 2020). These latters accumulate in fruits, flowers, roots and in the vegetative tissues of plants belonging to the Caryophyllales families, at the exception of the Caryophyllaceae and the Molluginaceae (Steglich and Strack, 1990). Their uniquiness is their N-heterocyclic nature derived from betalamic acid, which is their common biosynthetic precursor.

With a focus on food, epidemiological studies recommend a high intake of vegetables and fruits to improve global health and reduce free radicals' generation, associated with cellular and metabolic injury. Betalains are one of the most important natural antioxidants and food colorants. They are one of the earliest natural colorants developed for food industry (Francis, 1999). Several studies reported that red beet and cactus pear are good sources of natural antioxidants. The antioxidant activity of these pigments and its relationship with their molecular structures were previously detailed in the first part of this review (Chemical and Antioxidant Properties of Betalains). The annual production of betalains from cactus pear, red beet and pitaya fruits were estimated respectively at 18.7 tones (t) (Guevara *et al*., 2009), 96 794 126800 t (Pavokovic and Krsnik-Rasol, 2011) and 60.3 t (Vaillant *et al*., 2005; Le Bellec *et al*., 2006; Shea, 2012). In Tunisia, the annual production of betalains from spineless *Opuntia ficus-indica* was estimated at 543.33 t (Belhadj Slimen *et al*., 2020).

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Although numerous papers investigated the bioavailability and the health effects of betalains in humans, these research fields remain unexplored in farms animals. However, betalains may serve as strong natural lipid and vitamin stabilizers in animal feeds and may help to improve the overall livestock health. We reviewed previously the chemical and the antioxidant properties of betalains (Belhadj Slimen *et al*., 2017) and in this overview, the bioavailability of these pigmentsas well as the importance of their use as food additives in animal nutrition and health will be discussed.

Betalains in livestock nutrition

Changes in customer preferences towards more natural products and diet supplementation with natural bioactive compoundshave recently gained interest in animal feed research. Although betalains are health promising molecules, research has generally been conducted to human applications. Many aspects dealing with the metabolism, the bioavailability in livestock and the effect of these pigments on rumen functions need to be elucidated.

After the European ban of the use of feed antibiotics, the interest to develop natural feed additives able to protect against pathogens and to stabilize ruminal pH has increased (Chiquette, 2009; Meissner *et al*., 2010; Hutton *et al*., 2010, Shunmugadevi and Anbu Radhika, 2020; Atiba *et al*., 2021). In monogastric animals, plant derived alkaloids, such as sanguinarine, have been used as feed supplements in pig and poultry diets (Tschirner, 2004; Vieira, 2008). Alkaloids from *Sida acuta* was shown to reduce the growth of several gut pathogens, including those generating immune reactive LPS reactions (Karou *et al*., 2005). In ruminant models, plant derived alkaloids' supplementation was reported to decrease plasma lipoperoxidation (Gobert *et al*., 2009), modulate rumen microbial activity under stress conditions (Mickdam *et al*., 2016), improve crude protein degradation without ammonia release (Mickdam *et al*., 2016) and enhance feed intake with some improvement inaverage daily gain and feed efficiency (Yang *et al*., 2010).

Moreover, it is widely known that internal parasites are one of the greatest disease problems in grazing livestock worldwide (Waller, 2006). Since plant derived alkaloids and terpenes have antiparasitic properties (Kayser *et al*., 2003; Villalba *et al*., 2007), plants producing alkaloids as secondary metabolites may confer protection to grazing animals against these parasites. Consequently, betalains may be used as natural animal feed antioxidants.

Betalains' absorption and bioavailability

The bioavailability of a phytochemical is the percentage of the consumed compound that enters the bloodstream (Toutain, 2004; EAEMP, 2010). In betalain research, bioavailability assays were mainly conducted using *in vitro* models, rodents and human volunteers. Investigations are still far away from deciphering the route of betalain metabolism in ruminants. The obvious differences between ruminant and human's digestive systems may affect the bioavailability of these pigments and imply great differences in the absorptive and postabsorptive metabolism.

The intake of red beet juice or cactus pears revealed that maximum plasma concentrations of betanin and indicaxanthin are observed after 3 hours of ingestion and reached 0.6 μM and 6.9 μM respectively (Tesoriere *et al*., 2004a). Interestingly, these concentrations are higher than those reported for polyphenols (Holt *et al*., 2002; Schroeter *et al*., 2006). The half life of betanin is shorter than that of indicaxanthin (0.94±0.07 h vs 2.36±0.17 h respectively) and both compounds disappear from plasma after about 12 hours of intake (Tesoriere *et al*., 2004a). In urine, isobetanin was identified after 2-3 hours of red beet juice ingestion, leading to the suggestion of an isomeration of betanin to isobetanin, due to the relatively high temperature of the body (Jackman and Smith, 1996; Kanner *et al*., 2001).

In simulated gastrointestinal conditions, it was demonstrated that betalains are stable at pH below 3 and bile salt concentrations up to 4%. However, a decrease of their radical scavenging activity from 75% to about 38% was noted. The main loss (about 25%) was observed in the stomach conditions (Pavlov *et al*., 2005). Similarly, Tesoriere and co-workers reported a minor loss of indicaxanthin in the gastric-like environment and a decrease of vulgaxanthin I in all digestion steps. Betacyanins' loss was observed in the small intestine phase of digestion. The food matrix helped to prevent betalains' degradation marked by betalamic acid accumulation (Tesoriere *et al*., 2008). It can be concluded that the stability of betalains in the digestive tract depends on their bioaccessibility. Interestingly, betanin from red beet was reported to be less absorbed than that from cactus pear and to be significantly more bioavailable (Khan, 2016). This is probably due to the matrix effect.

Findings from *in vitro* studies suggest that betanin absorption takes place in the intestine (Neelwarne *et al*., 2013). It was also reported that betalains are absorbed in their intact form into the systemic circulation, since metabolic products such as betanidin and betalamic acid were not detected in plasma or in urine. These findings allow to suggest that hydrolysis and deglucosylation are not required for betanin absorption (Tesoriere *et al*., 2004a; Neelwarne *et al*., 2013). Tesoriere and co-workers investigated the intestinal absorption mechanism of indicaxanthin and betanin using Caco-2 cellmonolayers, which differentiate upon culture and become structurally and functionally similar to those of enterocytes (Turco-Liveri *et al*., 2007). This study ruled out that dietary indicaxanthin and betanin are absorbed through paracellular junctions of human intestinal epithelial cells. Indicaxanthin transport was shown to be non-polarized and function of time and concentration. The involvement of carriers and transporters in the pigment influx or efflux was reported. Nevertheless, betanin transport depends on its concentration and requires additional trans-membrane permeability. These findings are consistent with the higher bioavailability of indicaxanthin over that reported for betanin in humans.

As betalains are cationized compounds, they exhibit a high affinity for membranes (Kanner *et al*., 2001). They can bind to microsomes (Kanner *et al*., 2001) and they are partitioned within the lipid core of membrane phosphatidylcholine vesicles (Turco-Liveri *et al*., 2007). Absorption of betanin and indicaxanthin in red blood cells has been investigated and corresponding concentrations of 30 μM and 1 μM were reported (Tesoriere *et al*., 2005). It seems that the absorption mechanism was passive through paracellular diffusion, independent of carriers.

Betalains in animal health

Animal health is a major concern in animal production and since consumers are increasingly oriented to biological products, research has been focussed on natural pigments displaying biological effects.

Among stressors, oxidative stress was shown to reduce farm animals' welfare as well as production and reproduction performance (Nisar *et al*., 2013). Oxidative stress occurs when endogenous free radicals' generation overwhelms the antioxidant defense system, leading to lipid damage, protein synthesis inhibition, ATP depletion and DNA strand breaks (Turrens, 2003; Belhadj Slimen *et al*., 2014). Oxidative stress affects animal reproduction (Moreira da Silva *et al.*, 2010; Celi and Gabai, 2015), immunity (Sordillo and Aitken, 2009), growth (Avanzo *et al.*, 2001), milk production (Celi and Gabai, 2015) and meat quality (Archile-Contreras and Purslow, 2011). The most investigated causes of oxidative stress in veterinary medicine involve environmental factors such as high ambient temperature (Belhadj Slimen *et al.*, 2016), transport stress (Joachim *et al.*, 2010), dietary imbalances (Gabai *et al.*, 2004)) as well as inflammatory events such as mastitis, pneumonia, enteritis (Lykkesfeldt and Svendsen, 2007); parasitic infections (Celi, 2010) and negative energy balance during the post partum period (Castillo *et al.*, 2005; Pedernera *et al.*, 2010).

Antioxidant supplementation in livestock diets is a potentially important and affordable alternative to prevent from and to treat diseases related to oxidative stress, although its use remains controversial. Since betalains are natural antioxidants (Belhadj Slimen *et al.*, 2021), they could replace synthetic antioxidants used in farm animal feed. In fact, these pigments may allow protection of the feedfrom lipid peroxidation during storage and transport.Using electron spin resonance spectroscopy (ESR) and spin trapping, it has been shown that betanin acts as a scavenger of superoxide and hydroxyl radicals (Esatbeyoglu *et al.*, 2014). *In vitro* studies reported that these pigments protect LDL particles against oxidation at concentrations lower than those of α-tocopherol and catechin (Tesoriere *et al.*, 2003, 2004a; 2004b; Kanner *et al.*, 2001). Moreover, in human models, betalains were shown to decrease lipid oxidation biomarkers, including F_2-isoprostanes (in plasma), malondialdehyde (in plasma) and lipid hyperperoxide (in LDL) (Tesoriere *et al.*, 2003, 2004b). The anti-inflammatory activity of betalains was recently reported (Vidal *et al.*, 2014). Beetroot juice was shown to decrease LDH, ALT and AST levels (Szaefer *et al.*, 2014; Krajka-Kuźniak *et al.*, 2012). Interestingly, extracts frombeetroot decreased IL-6, TNF-α, MPO and NF-κB (El Gamal *et al.*, 2014). Phenethylamine-betaxanthin was shown to inactivate cyclooxygenase by interacting with Tyr-385 and Ser-530 close to the cyclo oxygenase active site, while betanidin was confirmed to inactivate lipoxygenase *via* interacting with the amino acids involved in substrate binding. Besides the inhibition/reduction of oxidative-induced DNA damage in neutrophils, lymphocytes and hepatocytes (in humans and rodents) (Lee *et al.*, 2009; Siriwardhana *et al.*, 2006; Zielińska-Przyjemska *et al.*, 2012), betalains were able to induce the endogenous glutathione synthesis in human erythrocytes (Tesoriere *et al.*, 2004b) and to protect red blood cells against hemolysis (Tesoriere *et al.*, 2005). Recent findings reported that betanin's antioxidant activity is due to its effect on the signaling pathway that mediates the transcription of antioxidant genes. Interestingly, betanin was shown to increase the activity of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which is a transcription factor that activates a gene promoter sequence: the antioxidant response element (ARE) responsible for the transcription of several endogenous antioxidant enzymes (Nguyen *et al.*, 2009; Krajka-Kuźniak *et al.*, 2013; Esatbeyoglu *et al.*, 2014; Satoh and McKercher, 2014). Besides inhibiting NADPH oxidase (NOX)-1 activation (Tesoriere *et al.*, 2014), *in vivo* trials confirmed betalains' ability to reduce oxidative stress and inflammation markers and to enhance antioxidant enzymes' activity (Martinez *et al.*, 2015). All of these trials are summarized in Table 1.

Betalain pigments can be considered as amphiphilic compounds since they possess hydrophilic groups (imino and alcohol groups) and lipophilic moieties (hydrocarbon chain). These chemical properties allow betalains to bind to membranes and to scavenge free radicals from the water phase and the propagating lipoperoxyls from the polar phospholipidic bilayer. Using a Gepasi modeling approach, the partition of indicaxanthin between water and vesicular pseudo phases was confirmed. Indicaxanthin can solubilize in the bilayer between the polar head groups and the hydrophobic region (Turco-Liveri *et al.*, 2009). Consequently, the interaction of indicaxanthin with membrane lipids may trigger anti-inflammatory and anti-apoptotic responses and protect from and treat related diseases. A proposed model of the interaction of indicaxanthin and betanin with cell membranes is represented in Fig 1.

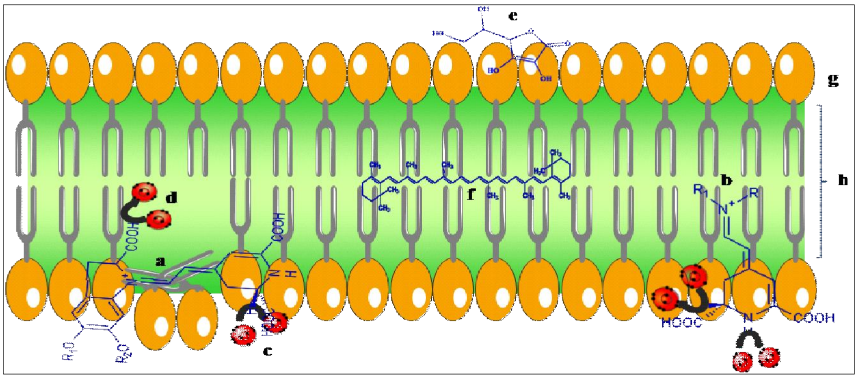

Fig 1: Proposed model of betaxanthins interaction with lipoperoxyl radicals and those of the water phase in phospholipid membranes compared to ascorbic acid and β-carotene.

(a) Betacyanin molecule (b) Indicaxanthin molecule (c, d) Free radical neutralization in both aqueous and lipid layers (e) Ascorbic acid molecule (f) β-carotene molecule (g) The hydrophilic part of the phospholipid (h) The hydrophobic part of the phospholipid.

Table 1: Effect of betalains on inflammation and oxidative stress markers.

Experimental model	Treatment	Administration mode and dose	Duration of the treatment (days)	Inflammation markers	Oxidative stress markers	Antioxidant enzymes	References
Wistar rats	Beetroot juice	Gavage 8 mL·kg·bm·day^{-1}	28	N/A	TBARS ↓ PC ↓ DNA damage ↓	SOD ↑ GPX ↑ CAT ↑ GR ↑	Kujawska *et al.*, 2009
ICR mice	Betalains from beetroot	Orally 0, 5, 20 and 80 mg·kg·bm·day^{-1}	30	N/A	MDA ↓	SOD ↑ CAT ↑ GSH ↑	Lu *et al.*, 2009
Wistar rats	Beetroot juice	Gavage 8 mL·kg·bm·day^{-1}	28	LDH ↓ AST ↓ ALT ↓	DNA damage ↓	GST ↑	Krajka-Kuźniak *et al.*, 2012
Osteoarthritic patients	Capsules made from beetroot extract	Orally 70-200 mg·day^{-1}	10	TNF-α ↓ IL-6 ↓ RANTES ↓ GRO-α ↓	AOPP ↓	N/A	Pietrzkowski *et al.*, 2010
Albino wistar rats	Beetroot pomace extract	Intraperitoneally 1-3 mL·kg·bm·day^{-1}	7	N/A	MDA ↓	GSH ↑ GSHPx ↑ GR ↑ CAT ↑	Vulić *et al.*, 2014
Albino wistar rats	Extracts of fresh beetroot	Orally 250 and 500 mg·kg·bm·day^{-1}	28	IL-6 ↓ TNF-α ↓ MPO ↓ NF-κB ↓	MDA ↓	CAT ↑ NP-SH ↑	El-Gamal *et al.*, 2014
Sprague-dawley rats	Beetroot juice	Gavage 8 mL·kg·bm·day^{-1}	28	LDH ↓ ALT↓	N/A	GST ↑ NQO1 ↑	Szaefer *et al.*, 2014

Betaxanthins may also serve as osmoregulators, since cacti contain high levels of proline. Interestingly, indicaxanthin is the major pigment in cactus pear and may induce osmoregulatory effects by modulating the amino acid pool due to betaxanthin cleavage or synthesis (Stintzing and Carle, 2004).

Indeed, due to their amine group, betalains may be involved in many biological processes, not only at the amino acid or protein level. Because of their hydrogen bonding properties, amines are much involved in binding polar molecules to macromolecules as in enzyme-substrate and hormone-receptor interactions. These interactions were reported for alkaloids, which are by definition natural nitrogenous compounds and some of which were shown to be involved in folk medicine and in natural biocides. In addition, as antioxidant plant compounds, betalains are able to activate the mammalian stress response and to induce heat shock proteins (HSPs) synthesis, which are considered as molecular chaperones involved in the repair of stress denatured proteins (Morimoto *et al.*, 1990; Hooper *et al.*, 2010).

Environmental-induced heat stress has adverse effects on livestock productivity (Das, 2018). Our previous studies showed that betalains ensure the thermoprotection of sheep lymphocytes by scavenging their H_2O_2 production level and preventing oxidative-induced apoptotic cell death (Belhadj Slimen *et al.*, 2019).

Finally, little is known about animal health benefits of betalains. Further investigations can broaden the hitherto narrow application area of these pigments.

Conclusion and Future Directions

This review presents a comprehensive summary of the relevant literature dealing with betalains' availability, discusses their importance in livestock health and proposes these pigments to be used as natural antioxidants and health promoting molecules in farm animal feed. Based on the literature sources related to betalains' stability, the addition step of these pigments during animal feed processing should be chosen carefully, in order to avoid thermal degradation. The hitherto available literature is far from giving valuable answers. Indeed, the stability of the supplemented animal feed during storage needs to be assessed. Although about 78 pigments were identified, bioavailability trials should propose the most interesting ones for commercial use. Contrarily to betaxanthins which diffuse easily across the cell membrane, the glucosylation of betacyanins induces their competition with dietary sugars and consequently a percentage of the absorbed pigments is discarded out of the cell. Indeed, there is a lack of data on the bioavailability of betalains once supplied to ruminant animals, their use by rumen bacteria, in addition to their ruminal stability and metabolism. Therefore, the microencapsulation of purified betalains may be an important research field in order to ensure their stability during animal feed processing and in the gastro-intestinal tract. More investigations are required to bring consensus to this.

Conflict of interest

The authors declare no competing financial interest.

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