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Nutritional and heat-protective characterization of Opuntia in semi-arid areas to mitigate heat stress in ruminant livestock systems.

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Abstract:

Due to its great adaptation to semi-arid and arid agro-climatic conditions, the Opuntia genus is gaining a great worldwide attention. The experimental plant material, evaluated by phytochemical analysis, consists of cladodes of Opuntia ficus indica f. inermis (OFI) and Opuntia megacantha (OM) species harvested in Mascara (located in the western region of Algeria). The results showed significant differences ($p<0.05$) between OFI and OM for all parameters measured. The relatively high humidity values of both species are directly linked to the crassulacean acid metabolism typical of cacti and the aridity effect of the environment. Crude protein values are moderately low in Opuntia cladodes of OFI and OM ecotypes, with 76.6 and 79.3 g/kg DM respectively. The fiber contents (NDF and ADF) of OFI and MO species vary from (314.4 and 118.2 g/kg DM) and (331.2 and 121.4 g/kg DM) respectively, with a superiority for MO. The highest levels of total polyphenols (TP) and flavonoids (FT) were recorded for OM cladodes (154.4 mg GAE/100 g DM and 4.45 mg QE/100 g DM) versus only (125.6 mg GAE/100 g DM and 2.26 mg QE/100 g DM) for the inerme form (OFI). Betalain compound values range from 4.65 to 8.54 mg/100 g DM for OF and OM, respectively, with betaxanthins predominating over betacyanins. Opuntia's richness in organic betaine and its electrolytic and osmolytic capacity represent a

solution for combating the risk of heat stress in livestock and maintaining the health and well-being of animals in the climate-constrained regions of developing countries.
Keywords: Nutritional attributes, bioactive compounds, opuntia, heat stress.

Introduction

Among of the most important challenges facing the ecosystems of the semi-arid regions of the southern Mediterranean region over the coming decades are the climate change and the scarcity of water resources. According to the IPCC, reports a significant global warming trend, with no increase in global surface temperature of 3.7 to 4.8°C, as well as in the frequency and intensity of extreme weather events such as heat waves, unless significant reductions in CO_2 and other greenhouse gas emissions are achieved rapidly (Belhadj slimen et al., 2016; Yerou et al., 2021).This global warming creates a state of heat stress for livestock, affecting productivity and income for medium and small-scale farmers. Indeed, in semi-arid regions, environmentally induced heat stress is a major concern, due to high ambient temperatures that compromise animal production (Das et al.,2016;Belhadj Slimen et al.,2016; Bellagi, 2017; Kumar et al.,2019; Yerou et al.,2021;Zoghlamiet al.,2022).Several research studies indicate that heat stress negatively affects most aspects of physiological functions (reproduction, growth, immunity, and animal health status) as well as product quality (Yasha et al.,2017; Wettere et al.,2021; Yerou et al., 2021; Zoghlami et al.,2022). Furthermore, the technical and economic profitability of farms is strongly influenced by the spread of the dry period and the threshold of heat stress expressed by the temperature-humidity index (THI), which exceeds the critical threshold THI>72. Consequently, nutritional strategies are needed to help animals reduce metabolic heat production and maintain production performance during heat stress (Belhadj slimen et al., 2015). Several strategies for mitigating the impacts of heat stress are proposed to combat the negative effects on breeding according to three basic management measures, physical modification of the environment, improving thermal tolerance through genetic modifications and improving diets (Yerou et al., 2021). Indeed, finding solutions based on the use of bioactive thermo protective compounds derived from plant bio resources, adapted to semi-arid climatic conditions, inexpensive and improving the health and well-being of livestock, is a major concern for the animal feed industry. In addition, to meet consumer demand for organic products, scientific research is focusing on natural pigments with biological effects on the product. The use of natural phytochemicals, in particular phytoantioxidants, are able to activate the stress response in mammals and induce the synthesis of heat shock proteins (HSPs), which are considered molecular chaperones involved in the repair of stress-denatured proteins (Hooper et al.,2010; Belhadj Slimen and al.,2019 and 2022). Several research studies indicate that betalains provide thermo protection for sheep lymphocytes by trapping their level of H2O2 production and preventing oxidation-induced apoptotic cell death (Belhadj Slimen et al., 2019; Garg, 2020).The global scientific community is currently showing great interest in plants whose metabolic pathway for carbohydrate synthesis is crassulacean acid (CAM) metabolism. This trend can be explained by the high tolerance of these plants to climate change, their ability to adapt to extreme conditions and their potential as a

valuable forage alternative in semi-arid ecosystems, where water is a limiting factor for livestock production (Abidi et al., 2009; Ammam et al., 2023). Livestock farming in semi-arid Mediterranean regions is particularly sensitive to heat stress due to high temperatures (Yerou et al., 2021; Zoghlami et al., 2022). The adoption of nutritional solutions to mitigate the harmful effects of heat stress on livestock is very necessary and represents added value for the valorization of bio resources in semi-arid zones. Opuntia, a shrub of the angiosperm dicotyledonous family Cactaceae, represents a good candidate for combating climate change and heat stress thanks to its nutritional composition and antioxidant properties (Belhadj Slimen et al., 2019; Medjekal et al., 2023; Ammam et al., 2023). This Opuntia shrub is widely distributed in North Africa particularly in semi-arid Mediterranean regions and possesses multiple medicinal, food, industrial and agro-ecological properties (Snyman, 2006; Belhadj Slimen et al., 2019; Ammam et al., 2023). Opuntia plantations are a valuable investment for certain arid and semi-arid regions of the world (Guevara et al., 2009). In Algeria, this species has a wide agro-ecological distribution and can be used in a variety of applications, such as soil erosion control, human and animal nutrition, and pharmaceutical industries and as a source of nectar for bee farms. The cactus shrub and fruit changed significantly as a function of climatic and edaphic environmental conditions in semi-arid conditions of Algerian. The cactus shrub reaches an equivalent production 10.23 kg (Douh and al, 2024). According to (Núñez-Gastelum et al.,2018; Belhadj Slimen et al.,2017) the profiling of the medicinal properties of Opuntia ficus-indica has revealed a myriad of phytochemicals with antioxidant and biological activities such as vitamins, phenolic compounds, flavonoids, carotenoids and betalains. Studies carried out in recent years have shown that fruits and cladodes contain a range of phytochemicals (phenolics, betalains) with bioactive properties (Belhadj Slimen et al., 2022).The objective of this research is the evaluation of the nutritional attributes and thermo protective properties of Opuntia in the semi-arid ecosystem of western Algeria, characterized by high temperatures for more than 5 months/ year, and to explore the possibilities of the nutritional solution based on bioactive compounds from Opuntia cladodes to mitigate thermal stress in livestock.

Materials and methods

Plant collection area

The substrates (cladodes) were collected in the region of Mascara (in north-western Algeria) located between longitudes 0° 55'E and 0° 15' E, and latitudes 35° 25' N and 35° 35' N. The climate, according to Koppen's classification, is semi-arid, with irregular rainfall averaging 350 mm per year. The choice of these shrubs is based on their availability, their high resistance to drought and their ability to produce biomass used in conditions of absolute soil marginality in the mountainous area of BeniChougrane, thus constituting a food resource for livestock. Identification of the two species was based on morphological traits (quantitative and qualitative) according to the classification criteria established by (Agüero et al., 2005; UPOV, 2006) table 1.

Table 1. Morphological identification of Opuntia ficus indica (OFI) and Opuntia megacantha (OM)

Parameters	OFI	OM
Cladodes Shape	Elliptical	Obovate to oblong
Length (cm)	7-63	30-60
Width (cm)	14-31	18-19.5
Thickness (cm)	1-3	1.5-2.5
Thorns	+ Absent	Present
Number/ areole	0-1	1-7
Length (mm)	3-10	20-35
Color	White	White or brown
Fruit		
Length (cm)	5-10	4.5-11
Diameter (cm)	4-7	3-4

Chemical and parietal composition

Cladodes were collected randomly from 10 plants per species during May and June. Cladode samples were washed, cut into small pieces and homogenized. Extraction with distilled water for 12 h was followed by centrifugation at 4500g at 4°C for 15 min. The supernatant was collected and stored at -20°C until use.

All assays are carried out in triplicate, and results are reported on the basis of 100g dry matter (% DM). The analyses carried out correspond to the determination of dry matter (DM) and mineral matter (MM) content in accordance with AOAC (1990) procedures. Crude protein CP was determined by the Kjeldahl method. Parietal constituents (NDF: Neutral Detregent Fiber; ADF: Acid Detregent Fiber; ADL: Acid Detregent Lignin) were determined using the method of Van Soest et al., (1991).

Determination of secondary compounds

Total phenolic compound (TP) content was estimated according to Ainsworth and Gillespie (2007) and expressed in gallic acid equivalents (GAE). Total flavonoids (FT) were determined according to the method described by (Zhishen and al., 1999) and expressed in quercetin equivalents (QE). Betalains were determined according to the procedure of Prakash and Manikandan (2012). Betalain content was assessed according to the equation of Stintzing et al., (2005):

$$BC\ (mg/L) = (A * DF * MW * 1000) / \varepsilon * l$$

With: BC: betacyanin or betaxanthin content, A: sample absorbance, DF: dilution factor, MW: molecular weight (indicaxanthin = 308 g/mol and betanin = 550 g/mol), ε: molar extinction coefficient (indicaxanthin = 48,000 L mol-1 cm-1 at 480nm and betanin = 65,000 L mol-1 cm-1 at 536 nm), l: cuvette path length (cm).

Statistical analysis

All data were subjected to statistical analysis of variance using SPSS software, with the model: $Yij = \mu + Si + Eij$ Where Yij represents the observation of the dependent variable, μ the population mean for the variable, Si the species effect and Eij the random effect associated with the observation.

Results and discussion

Chemical and parietal composition

Analytical results for the chemical composition of (OFI) Opuntia ficus indica and (OM) Opuntia megacantha are shown in Table 2. Cladode moisture contents were 84.3% and 85.6% for OFI and OM cladodes respectively. The relatively high humidity values of both species are directly related to the crassulacean acid metabolism typical of cacti and the aridity effect of the environment. Statistical analysis of the results shows significant differences ($p < 0.05$) between the two species studied for all parameters measured. The dry matter content of thorny species (OM) is slightly higher than that of (OFI). Total nitrogen values are moderately low in opuntia cladodes for both the inerminous (OFI) and thorny (OM) varieties, at 76.6 and 79.3 g/kg DM respectively. Parietal composition for the NDF and ADF fractions for OFI and OM species varies from (314.4 and 118.2 g /kg MS) and (331.2 and 121.4 g /kg MS) respectively, with superiority for OM.

Table 2. Chemical and parietal composition of shrubs (g / Kg DM)

Parameters	Opuntia ficus indica (OFI)	Opuntia megacantha (OM)
Hd %	84,3 ^{a}	85,6 ^{a}
MM	289,6^{a}	293,3^{b}
CP	76,6^{a}	79,3^{b}
CB	100,2^{a}	110,3^{b}
NDF	314,4^{a}	331,2^{b}
ADF	118,2^{a}	121,4^{b}
ADL	18,2^{a}	20,8^{b}
Hd in % : moisture ; MM : mineral matter ;CP : crude protein ;CB : crude cellulose ;NDF: neutral detregent fiber ;ADF : acid detregent fiber ;ADL : acid detregent lignin ; on the same line, values bearing a different letter are comparable at the 5% threshold.		

Phytochemical characteristics

The antioxidant properties of polyphenols are highly sought-after for alleviating oxidative stress. The abundance in OFI and OM cladodes could be used to combat heat stress in livestock in areas where heat stress exceeds the critical threshold. The results indicatethat highest total polyphenol (TP) and flavonoid (FT) contents were recorded for OM cladodes (154.4 mg GAE/100 g DM and 4.45 mg QE/100g DM) compared with OFI (125.6 mg GAE/100 g DM and 2.26 mg QE/100g DM).Betalain content results vary from 4.65 to 8.54 mg/100 g DM for (OF) Opuntia ficus indica and (OM) Opuntia megacantha, respectively with a superiority of betaxanthins over betacyanins table 3.

Table 3. Phytochemical composition of the cladodes Species

Parameters	Opuntia ficus indica (OFI)	Opuntia megacantha (OM)
PT	125,6^{a}	154,4^{b}
PT	125,6^{a}	154,4^{b}

FT	2,26[a]	4,45[b]
BT	4,65[a]	8,54[b]
PT: total phenols (mg gae/100 g ms), FT: total flavoniodes (mg qe/100 g ms), BT: betalains (mg/100 g ms). On the same line, values bearing a different letter are comparable at the 5% threshold.		

Currently, there is a growing interest in nutritional and therapeutic plant-based solutions that can mitigate the damaging effects of heat stress. In many cases, substances of plant origin and herbal preparations successfully complement conventional breeding practices in hot climates without causing side effects. The Opuntia genus is considered an excellent natural biomass. It is a fast-growing xerophilous plant, resistant to draughts and well adapted to a hot, arid environment (Sahoo et al., 2017). It is important to find animal feed alternatives to make its use more efficient in a sustainable livestock production context. Opuntia can be considered an excellent and cheap source for diet supplementation, a versatile crop and an alternative feed (Nazareno., 2017; De Oliveira et al. 2017;Cardosoa et al., 2019; Hassan et al., 2019), due to its efficiency in converting water into dry matter. In addition, this species has high levels of betalains, total carotenoids, ß-carotene and ascorbic acid, and is one of the best sources of total phenols.Indeed, nutritional and health opportunities are linked to its antioxidant properties due to ascorbic acid, polyphenols, flavonoid compounds and the mixture of yellow betaxanthin and red betacyanin pigments (Zenteno-Ramírez et al., 2018).

Moisture content results for OFI and OM are lower than those reported by Stintzing and Carle (2005) for Opuntia spp cladodes (88% and 95%) and by Harrak (2021) for cladodes of Opuntia ficus-indica species. The ash content for OFI and OM are higher than those reported for the OFI species in the semi-arid zone of eastern Algeria by Medjekal et al.,(2023) and for some cultivars in Morocco (Loukhmas et al.,2020 and 2021, Astello-García et al., 2015) for the same species studied Opuntia ficus-indica and Opuntia megacantha. This richness is explained by the presence of crystalline structures formed by calcium oxalate on the majority of epidermal cells and some parenchymal cells (Reale et al., 2016). Similar values were reported by Medjekal et al.,(2023) for OFI in the semi-arid zone of eastern Algeria, and (Abidi et al2009, Andrade-Montemayor et al.,2011), while higher results were reported by (Ayadi., 2009) in Tunisia and for varieties grown in Mexico (Rodriguez-Felix et al.,1988).

Several studies have reported variations in the crude protein (CP) content of Opuntia ficus indica ranging from 3 to 12.8% (Misra et al., 2006; Tegegne et al., 2007). The crude protein contents of OFI and OM are in the minimum range (7-8% DM) required for rumen function to ensure maximum metabolic activity and proper feeding of small ruminants (Van Soest., 1994; Boufennara et al., 2012). Ruminant intake decreases when the CP content of the feed falls below the threshold of 6-8% DM, resulting in insufficient microbial growth in the rumen, which is not conducive to rapid forage degradation. This deficit can be corrected by adding various nitrogen sources, such as Atriplex halimus, which is present in the study area. Moreover, CP content should not be the only criterion for judging cactus characteristics. Indeed, some authors indicate that the reactivity, structure, molecular weight of plants and the interactions of their various secondary

metabolites are more important than the levels contained in determining the suitability of a given plant species as a protein supplement. The difference in fiber content (NDF and ADF) between the two species can be explained by the behavior and plasticity of each species according to the edaphic and climatic conditions of the region. These results should be taken into consideration when using cactus in livestock feed. According to Ben Salem et al (2002), the use of cactus cladodes in rations should be combined with other forages, as the ingestion of cladodes alone leads to diarrhoea, as the animals' digestive transit speed increases.

The phytochemical content of opuntia cladodes, particularly those with antioxidant potential, could be used as feed supplements to improve the quality of formulated diets and reduce the risk of developing resistant bacteria, as well as to improve animal performance (Lai and al., 2018, Correddu and al., 2020; Morshedy and al., 2020). In addition, dietary use of natural antioxidants is the best way to control oxidative stress, minimize free radical production and improve animal health (Abuelo and al., 2019).Indeed, phenolic compounds are generally classified as typical functional components of the plant cell wall, and they play a key role in defense mechanisms against most abiotic stresses, including UV irradiation, water stress, and higher temperatures. These polyphenol results are comparable to those reported in Morocco 133.9 and 165 mg GAE /100 g MS for Opuntia stricta (Lahmidi et al., 2023). Furthermore, the polyphenol contents found are higher than those reported by Elshehy et al.,(2020) for Opuntia ficus-indica cladodes (119.66 mg/100 g DM) and lower than those reported by (Loukhmas et al.,2020 and 2021). However, the total flavonoid content is comparable to that of Opuntia megacantha in southern Morocco with 5.02 mg EQ/100 g FM (Bouzoubaâ et al., 2016).

Metabolic profiling of Opuntia ficus indica epidermal and mesophyll tissues revealed that flavonoids were higher in plants exposed to water stress (Mayer et al., 2021). Lack of water induces the generation of reactive oxygen species, which cause oxidative damage to the plant. These differences with our results could be attributed to different cultivars, geographical origin, growth stage of cladodes as well as extraction protocols and analytical assays. Analysis of the phenotypic diversity of Opuntia ficus-indica in Algeria showed that there are variations between species at spine and areole level that appear to be a response to climatic variations. According to Díaz and al (2017) Cactus, as an agricultural byproduct, is rich in betalain pigments, which a mixture of yellow betaxanthin and red betacyanin, which are excellent radical scavengers with an antioxidant activity higher than ascorbic acid, rutin, and catechin. Additionally, cactus contains phenolic compounds, flavonoids, total carotenoids such as beta-carotene, vitamin E and other antioxidants such sterols, esters, saponins and alkaloids (Yahia et al., 2017; Zenteno-Ramirez and al., 2018; Tahir and al., 2019). As a result, many research studies report that opuncia has proven benefits for boosting immunity and general health (Díaz and al., 2017; Tahir and al., 2019). In addition, this species also possesses several highly interesting biological properties, namely anti-inflammatory, antimicrobial and antiviral actions (Morshedy and al., 2020). However, research into the use of opuncia as a potential feed supplement for farm animals due to its high phytochemical content is still insufficient to characterize the real potential of this species.

Improving performance, animal health and welfare is a major concern in the animal industry, and in response to consumer demand for organic products, scientific research is focusing on natural

pigments with biological effects. According to Belhadj Slimen et al., (2022), oxidative stress affects the well-being of farm animals, as well as their production and reproductive performance. In semi-arid and arid southern Mediterranean ecosystems, the breeding environment is characterized by high ambient temperature and thermal stress exceeding the threshold of thermal neutrality, causing oxidative stress. The use of natural antioxidants in animal feed is a potentially important and affordable alternative for preventing and treating diseases linked to oxidative stress. Betalains, being natural antioxidants, could replace synthetic antioxidants used in livestock feed (Belhadj Slimenet al., 2021).

Betalain values in Cactaceae show wide variation depending on species and agroecological zones (Bouzoubaâ et al., 2016; Du Toit et al., 2018; Elshehy et al., 2020; Mahdeb et al., 2021; Gómez-López et al., 2021; Belhadj Slimenet al.,2022; Lahmidiet al., 2023).In a study conducted by Belhadj Slimen et al (2019) on the effect of betalain on the thermoprotection of Tunisian sheep lymphocytes the results indicate that the use of OFI opuntia extracts prevents oxidation-induced apoptotic cell death. Indeed, the result on the ability of cladodes extracts to reduce oxidative stress induced by various oxidizing agents, and the protection of lymphocytes against heat-induced mortality open up possibilities for the use of opuntia bioactive molecules to reduce heat stress in arid areas by (Akacha et al., 2015; Belhadj Slimen et al., 2019).Further research may broaden the scope of phytochemical properties, including opuntia betaines, as additives in livestock rations.

Finally, betaine of organic origin is a potential electrolyte and osmolyte with an osmotic function for the epithelial cells and microflora of the digestive tract. It represents a solution for combating ST and could help ruminants and monogastrics such as poultry and rabbits on small rural farms in developing countries suffering from heat stress to maintain their health status while preserving their performance.

Conclusion:

The physicochemical evaluation of Opuntia ficus indica and Opuntia megacantha cladodes reveals the nutrient richness of both species. OM cladodes are superior to OFI. Values for bioactive compounds showed high betalain thresholds. Betalains ranged from 4.65 to 8.54 mg/100 g DM for (OF) and (OM), respectively, with betaxanthins being superior to betacyanins. These natural antioxidants could replace the synthetic antioxidants used in livestock feed. In fact, betalains in Cactaceae vary widely according to species and agro-ecological zones. The high polyphenol, flavonoid and betalain content of cladodes from local ecotypes in semi-arid zones indicates that cladode extracts can be used and valorized as sources of natural antioxidants based on bioactive opuntia molecules to reduce heat stress in arid zones. Thus, planting these species as crops or as part of projects to restore semi-arid zones will contribute to improving the environment and the livelihoods of the local population.

Our guidelines and recommendations for the use of opuncia cladodes require experimental trials on indigenous livestock reared by farmers in the study area, to determine the thresholds of use and effective doses required for the formulation of feed rations. Further research will also be

carried out into the possibilities of introducing cladode powder into the cattle feed manufacturing industry.

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