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climate classification)) [2-4]. In Mexico, HS production has increased 10.54% from 2003 to 2018 [5, 6].

According to FAO [7], the HS calyxes are one of the most demanded products by industry to human feeding [8, 9]. Due to fatty acids HS contents and proportions [10, 11], antioxidants [12-14] and antimicrobial (Gramm negative bacteria) [15], antiviral [16], and anthelmintic properties [17], which might have improvements on human wealth [12, 18, 19].

Calyxes of HS contain 15.76-0.04% of a-linoleic fatty acid (n-3) [10, 11], and flavonoids classified as anthocyanins [20]. Factors as the type of HS variety, crop management, processing, storage, extraction of extracts, and cell contents affect the antioxidant contents [21-23], however, the most proportion of HS flavonoids are the anthocyanins cyanidid-3-O-sambubioside (25.9 to 46.2%) and delphinidin-3-O-sambubioside (48.4 to 59.2%) [13, 23-25] whose clinic effects on humans are different from another kind of flavonoids supplements as green tea (Camelia sinensis) which mainly contain epigalocatequin-3-gallate (EGCG) and epicahequin-3-galllate [26].

Overall polyphenols and another kind of antioxidants as selenium and a-tocopherol reduce the free radicals and chelate pro-oxidant metals [9, 14, 27-29], can affect the ruminal digestibility and fermentation kinetics [30, 31], and in the animal productive behavior [32], reducing the effects of the oxidative stress in ruminants [33-35] caused by the high grain and concentrates proportions on diets [26, 36, 37], the metabolic exigency, and the heat stress [37], and therefore improving the oxide-reductive potential of products derived to human feed [38].

The objective of the present study was to make a critical review about the potential clinic and productive effects of including HS anthocyanin: cyanidin-3-O-sambubiosid and delphinidin-3-O-sambubiosid in ruminant diets.

2. Oxidative stress in ruminants

Inflammatory and environmental processes increase the endogenous ROS. Unbalance between pro-oxidants and antioxidants might promote oxidative stress and molecular damage [37].

In dairy cows and beef cattle, the environmental pollution and the high metabolic exigency during pregnancy, milk production, negative energy balance, heat stress, respiratory diseases, inflammatory process, and parasites promote ROS releasing (O₂, OH, RO₂, RO, HO₂, H₂O₂, HOCI, O₃, etc.), meanwhile the adipose mobilization increases the pro-inflammatory cytokines [37]. Potential negative effects on animal wealth would worse in the future because of the population increment and therefore the milk and meat demand [27].

ROS contribute to inflammatory processes through the necroptosis activation (NF-κβ) via phosphorylation interleukin (I-κβ), and because of the production of pro-inflammatory cytokines such tumoral factors (TNF-α). In addition, protein carbonylation is mediated by the ROS and metals (Fe²⁺, Cu⁺, etc.), producing oxidative by-products and advanced oxidative protein products (AOPP): 1) carbohydrates and lipids have reactive compounds to carbonyl from glycoxidation and lipoperoxidation that might bond to protein residues; 2) oxidized proteins are degraded by proteases, but chemically modified proteins (by di-tyrosine and disulfide cross linkages) might not be substrates to proteolysis, contributing to deposits in tissues and organ damages [39-44].

High grain and concentrates proportion in ruminant diets increases the lipoperoxidation, decreasing the α-tocopherol and the ferric reductive availability in blood plasma [22] and increasing the amount of AOPP, negatively related with milk yield because of the oxide-reductive unbalance. Including high-grain diets and therefore the reduction of forage proportion rises the abnormal amount and types of metabolites in rumen [37].
In viral, bacterial, and fungal infections, phagocytes and neutrophils are sources of ROS that interfere in a chain of chemical reactions that increase the hypochlorous oxidant potential which might be useful to combat the photogenes, but damaging tissues. Besides this, parasites induce inflammatory followed by eosinophils increasing which also contribute to tissues damage. Lactation and heat stress are potential sources of AOPP, thereby of TNF-α expression and potential mammal glandule diseases, increasing the milk and meat contents of ROS [45, 46].

Oxidized milk and meat contribute to a higher ROS content in blood plasma which would be a threatening to human welfare [38].

3. Potential clinic effects of antioxidants

Polyphenols are a wide variety of secondary plant metabolites with at least one -OH that can be structurally simple (gallic and gallic acids) or complex (dymers, olygomeric, and polymeric with high molecular weight). Antioxidants can be classified as flavonoids or non-flavonoids, thus flavonoids can be flavones, flavanones, isoflavones, flavonols, flavan-3-ols and anthocyanins (from flavan-3-ols derived the condensed tannins (non-hydrolysable)), phenolic acids, hydrolysable tannins, and stilbenes are clustered as non-flavonoids [17].

Because of the structural differences among complexes, total phenolic compounds cannot directly be related with total antioxidant availability [21, 22]. The EGCG, primarily found in green tea had a galloyl group in the third position and an o-trihydroxy in the β-ring which protect cell from ROS damage [42, 47]; by the regulation the over expression of genes EGCG have anti-inflammatory and antioxidant effects in reduction of apoptosis, cell fibrosis, and tumoral growing via regulation and reduction of kinases, signal transduction, and transcription activation [44, 48]. EGCG can [49]:

1) Promote the cytotoxicity to increase the antitumoral activities, by producing H2O2 with its pyrogallol moiety or the reduction of Fe(III) to Fe(II), generating -OH ROS (although cysteine N-acetyl protect cells from cytotoxicity of H2O2 it does not avoid cell death process).

2) Promote apoptosis through mitochondrial damage, membrane depolarization, and cytochrome c release, and protects against mitochondrial damage-related cell death without changes in SOD, glutathione peroxidase, Nrf2, Bcl2, and oxidative stress. Modulates gene expression by inhibiting various transcription factors (including Sp1, NF-κB, AP-1, STAT1, STAT3, and FOXO1) and the expression of NF-κB and AP-1. EGCG inhibits STAT1 to mediate protective effects on myocardial injury.

3) By increasing second messengers, such as Ca\(^{2+}\), cAMP, and cGMP. EGCG elevates cytosolic Ca\(^{2+}\) without electrical stimulation by inhibition of SERCA (Ca\(^{2+}\)-ATPase activity), which affects the activities of Ca\(^{2+}\)-requiring enzymes, such as calmodulin (CAM)-dependent protein kinase II and CAMKK\(^{\beta}\) (CAMKK\(^{\beta}\) is an upstream regulator of AMP-dependent kinase (AMPK), which plays crucial roles in energy metabolism and cardiovascular functions). Stimulates vasorelaxation by increasing cAMP and cGMP in the aorta, then it may stimulate the production of cyclic nucleotides with beneficial biological effects in cardiovascular physiology.

4) Inhibit the transcription of FOXO1 to lead the suppression of basal levels of endothelin-1 and differentiation of adipocytes. In mitochondria, EGCG enhances fat utilization, reducing the expression of leptin and stearyl-CoA desaturase while increasing fat oxidation.

5) EGCG inhibits DNA methyltransferase, which reverses methylation-induced gene silencing.

6) Inhibits autophagy, leading to apoptosis in macrophage cell lines.

Although the extracts of HS also change the oxidative potential of blood plasma, increasing the glutathione intracellular, but its primarily action is on Renin-Angiotensin-
Aldosterone System (RAS) interfering the electrolytic regulation, blood pressure, and the cardiac function [50], the increasing of adrenalin, catecholamines, and noradrenalin (by specific Angiotensin (AngII)) [51].

Guerrero et al. [52] tested the activity of the Angiotensin Converting Enzyme inhibitor (ACEi) of 17 different types of flavonoids, the ACEi increased when: 1) the catechol group was in the β-ring (3’, 4’-dihydroxy); 2) there is a doble bond between C2 and C3 of carbon rings; and 3) there is a ketone in the C4 of the carbon ring. The absence of C4 in the carbonyl group of EGCG reduce the ACEi ability, delphinidins-3-O-sambubioside and cyanidin-3-O-sambubioside chemical structures have primarily ACEi potential.

Studies included in vivo cells [50] showed that delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside inhibit 43 to 50% the ACE (delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside vs. control, and 30% less than captopril), furthermore, anthocyanins interfered in the RAS reductive process (RT-qPCR mRNA of ACE and renin were analyzed), reducing 37 to 52% the rARN expression for renin. To test the clinic effect of anthocyanins of HS, Nurfaradilla et al. [53] blocked the left renal artery of mice (2K1C hypertension) and treated them with HS extracts (30 mg/200 g BW), captopril, and captopril+HS mixtures; HS extracts reduced the systolic blood pressure 17% (average 150 vs. 88, and 80, control vs. HS, and captopril), although captopril and HS reduced the renin ad AngII in plasma, HS reduced the ACE activity (1.5 µmol/mL/min control vs. 0.40 µmol/mL/min HS, vs. 0.30 µmol/mL/min captopril).

Other potential pharmacological properties of HS antioxidants are anti-hypercholesterolemia, antipyretic, antibacterial, antiviral, and anthelminthic [13, 54].

4. The effect of the anthocyanins in ruminant diets and their productive behavior

4.1. Effects of anthocyanins in ruminal digestibility, volatile fatty acids, and potential methane gas emissions. The ability of antioxidants to maintain its activities in ruminal environment, and the molecules abilities to reach the bowel without major modifications. Although some in vitro studies show no differences among ruminal gas production and degradability [30, 31, 55], however anthocyanins can improve the ruminal antioxidant potential [30, 31]. Some flavonoids (e.g. tannins) have effects on ruminal microbiota [17, 45], modifying the gas production kinetics and the volatile fatty acids (VFA) proportions.

The chemical structure, distribution, and elimination of flavonoids affect the interaction and/or synergism between them and the ruminal microbiota. Although all the effects of anthocyanins in rumen remain unclear, antioxidant and antimicrobial activities of HS are related to the reduction of methane and N-ammonia (CH$_4$ and NH$_3$) caused by the changes of the by-products that affect the methanogenic microorganisms growth [45, 47].

Some no desirable antioxidant effects are the reduction of the endogenous fibrolytic enzymes activities, and thereby the potential fiber digestibility and protein absorption [57]. In addition, as other polyphenols sources, some HS components with high-lignin contents have low DM digestibility (DMD), however, antioxidants can modify and improve the biohydrogenation of fatty acids [57] and increase the milk and meat polyunsaturated fatty acids (PUFA) [58].

4.2. Effect of anthocyanins after rumen. Some polyphenols are hydrolyzed and transformed through endogenous enzymatic activities and ruminal bacteria [59], therefore the secondary metabolites cross through the ruminal epithelium and the non-absorbed are bio-converted in the small bowel (as it occurs in monogastric) [59] and pass to the bloodstream [34, 57, 60, 61] to deposit in tissues [45, 60].

Anthocyanins can improve the blood plasma resistance to oxidation [32, 62]. Cyanidin-3-O-sambubioside and delphinidin-3-O-sambubioside can be deposit in lung, cardiac, renal, and hepatic tissues [46], suggesting that anthocyanins can improve the meat and milk antioxidant potential. In addition to the improvement of biohydrogenation of
fatty acids in the ruminal environment, anthocyanins increase the animal products to human feed.

Although the milk yield and fat milk have improvements have not been related to anthocyanin addition in ruminant diets [32], the potential clinical effects of delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside of HS on RAS, could as in humans, interfere in the homeostatic balance of ruminants affecting the milk yield [63].

Although the reports about the potential effects of HS anthocyanins on fertility parameters are not consistent, other sources of polyphenols, as coffee can improve the semen quality [64] but could reduce the fertilization rates even when progesterone, estradiol, and follicle-stimulating hormone (FSH) remain constant [61]. In contrast, other types of antioxidants as selenium and α-tocopherol might increase some reproductive parameters [27]. Therefore, further studies could be focused on the effect of HS anthocyanins on estrous, and milk and meat production.

5. Effects of HS anthocyanins milk and meat shelf-life

Besides the positive effects of increasing the meat and milk antioxidants on human welfare, anthocyanins could increase the shelf-life of animal products [65-67]. Overall, polyphenols avoid lipids and proteins oxidation (hyper-peroxides, aldehydes, and ketones), autolysis, and microbial pollution [28, 68-70].

6. Hibiscus sabdariffa L. by-products

As with other agricultural wastes and by-products the inclusion, of the seeds, stalks, and leaves of HS might reduce the economic and environmental livestock costs [71-73], in addition, optimal inclusion of by-products and wastes in balanced ruminant diets should not have negative effects on animal productive behavior [67, 74-76].

The phenolic and antioxidant activities of HS seeds have been previously assayed and resulted similar or better than those in calyces [77], but the comparison of the potential effects of seeds with calyces should be assayed in ruminal liquid, including a test to interpret the ruminal microorganisms-fibrolytic enzymes with the feedstuff' cell walls.

In average, HS seeds have: crude protein (CP), 27.9±10 g/100 g of dry matter (DM); fat, 18.8±8.6 g/100 g DM; crude fiber (CF), 16.8±11.1 g/100 DM; and ashes, 5.86±3.2 g/100 DM (74, 78) (Table 1).

<table>
<thead>
<tr>
<th>Authors</th>
<th>DM g/100 g</th>
<th>CP g/100 DM</th>
<th>EE g/100 DM</th>
<th>CF g/100 DM</th>
<th>Ashes g/100 DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maffo et al. [74]</td>
<td>90.0</td>
<td>22.0</td>
<td>22.0</td>
<td>20.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Wang et al. [88]</td>
<td>NR</td>
<td>NR</td>
<td>18.0</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Ismail et al. [89]</td>
<td>90.0</td>
<td>33.5</td>
<td>22.1</td>
<td>18.3</td>
<td>NR</td>
</tr>
<tr>
<td>Shaheen y El-Nakhlawy [90]*</td>
<td>NR</td>
<td>31.4</td>
<td>23.2</td>
<td>4.29</td>
<td>5.5</td>
</tr>
<tr>
<td>Udayasekharra [91]**</td>
<td>92.4</td>
<td>20.6</td>
<td>21.0</td>
<td>41.1</td>
<td>5.4</td>
</tr>
<tr>
<td>Beshir y Babiker [84]</td>
<td>96.6</td>
<td>30.3</td>
<td>11.1</td>
<td>5.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Fagbmho [2]</td>
<td>92.6</td>
<td>39.4</td>
<td>6.1</td>
<td>17.7</td>
<td>11.4</td>
</tr>
<tr>
<td>Jinez et al. [92]</td>
<td>92.5</td>
<td>20.6</td>
<td>18.0</td>
<td>23.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Kwari et al. [93]</td>
<td>NR</td>
<td>38.6</td>
<td>NR</td>
<td>13.5</td>
<td>NR</td>
</tr>
<tr>
<td>Mukhtar [94]</td>
<td>91.8</td>
<td>21.4</td>
<td>17.4</td>
<td>12.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Soriano y Tejeda [95]</td>
<td>92.7</td>
<td>24.8</td>
<td>17.8</td>
<td>22.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Table 1. Hibiscus sabdariffa L. chemical composition.
Anhwange et al. [96] 94.0 19.8 28.0 6.3 5.6
Tounkara et al. [81] 91.8 27.3 20.8 NR 4.5

DM, dry matter; CP, crude protein; EE, ether; CF, crude fiber; NR, no reported; *Average of three varieties; **Average from two varieties.

CP content of HS seeds is comparable to the soybean and canola seeds (79), their fatty acids are primarily oleic and linoleic (n-9 and n-6) (37.68±1.10% and 34.14±1.25%) (Table 2) (10, 80, 81), and its DM and CP in situ degradability had been similar to sunflower and peanut seeds [82].

Table 2. Proportion of fatty acids in Hibiscus sabdariffa L. seeds and calyxes.

<table>
<thead>
<tr>
<th></th>
<th>Seeds</th>
<th>Calyxes</th>
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<tbody>
<tr>
<td>Saturated fatty acids (%)</td>
<td></td>
<td></td>
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<tr>
<td>Miristic (C14:0)</td>
<td>0.21</td>
<td>0.26</td>
</tr>
<tr>
<td>Palmitic (C16:0)</td>
<td>19.21</td>
<td>20.52</td>
</tr>
<tr>
<td>Estearic (C18:0)</td>
<td>5.13</td>
<td>5.79</td>
</tr>
<tr>
<td>Araquidonic (C20:0)</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Polunsaturated fatty acids (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitoleic (C16:1)</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Oleic (C18:1)</td>
<td>36.9</td>
<td>38.46</td>
</tr>
<tr>
<td>Linoleic (C18:2)</td>
<td>35.02</td>
<td>33.25</td>
</tr>
<tr>
<td>α-linoleic (C18:3)</td>
<td>1.85</td>
<td>1.69</td>
</tr>
</tbody>
</table>

Substituting 75% of total CP might not negatively affect animal performance [78]. Previously, the inclusion of ≤25% of the total DM of sheep’ diets with HS seeds increased the final body weight and carcass proportion [83], and in other studies, adding 10-20% of HS seeds improved the organoleptic and quality fatty acids properties of sheep meat [84].

Overall, increase the long-chain fatty acids (n-3, n-6, and n-9) in ruminant diets improve the fatty acids composition of milk and meat [85-87].

7. Limitations and perspectives

The relationship among potential antioxidant activities of calyxes, seeds, and stalks anthocyanins of HS with the ruminal microbiota and fibrolytic enzymes remain unknown. In comparison to the studies included in the present review that tested other polyphenols in the ruminal environment, hypothetically positive effects of HS anthocyanins would be the potential reduction of CH₄, and on the fatty acids biohydrogenation process, but also it could reduce the potential fiber degradability [45, 47, 57, 58].

Since antioxidants have a potential reduction of AOPP related with the milk yield improvement, the available information about biochemical and RAS changes promoted by delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside of HS [52, 63] could be considered in further in vivo studies to find inclusion doses that would improve the composition of the antioxidant and fatty acids and milk yield. However, optimal inclusion should avoid potential negative effects on animal performance and reproductive parameters.
8. Conclusions

The excess of ROS unbalances the oxide-reductive potential in ruminants fed with high-grain diets, exposed to bacterial, viral, and helminthic diseases, and to excessive metabolic exigency and heat stress. HS contain flavonoids primarily classified as anthocyanins which are mainly cyanidin-3-O-sambubioside and delphinidin-3-O-sambubioside show specific actions on RAS regulation, increasing the ACEI action and reducing the expression of renin genes. In ruminal environment, they can reduce methanogens microorganisms, and interact with substrates, fibrolytic microbiota and enzymes affecting the fiber degradability and the lipids biohydrogenation, which might change the animal performance and the quality of milk and meat lipids. After rumen, anthocyanins are absorbed in small bowel and cross to bloodstream improving the blood resistance to oxidation, and they can be deposited in tissues to increase the milk and meat yields and antioxidant potential. Further studies about the specific action of cyanidin-3-O-sambubioside and delphinidin-3-O-sambubioside on RAS in ruminants would be useful to understand their potential effects on milk yield, besides this, HS antioxidants should be analyzed on the ruminant reproductive parameters. Although the HS seeds antioxidant effects remain unknown, including HS seeds had not negative affected the ruminant productive behavior but had improved the body weight gain and the fatty quality and proportion in meat.

Author Contributions: Present study was conceptualized, designed, and directed by D.N. Tirado-González. Although all authors were actively involved in data collection, analysis, and discussion process, R. Lazalde-Cruz and M.I. Carrillo-Díaz were responsible to inclusion criteria; L.A. Miranda-Romero and G. Tirado-Estrada were responsible of the formal analysis of results; G.D. Mendoza-Martínez, and A. Lara-Bueno analyzed the validity of derived conclusions; D.N. Tirado González, visualization, and wrote the draft paper. All authors reviewed and edited the final version and have read and agreed with the final decision of submitting and publishing the manuscript.

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References


