Astaxanthin – King of antioxidants as immune modulator and anti-inflammatory for enhancing productive performance and health of animals

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Abstract: Astaxanthin is the potent antioxidant predominantly found in living organisms that are present in marine environment. It is known as “King of antioxidant” or “Super vitamin E” because of its potent antioxidant property. Antioxidant property of astaxanthin known to modulate different biological activity relating to antioxidant defence system, inflammation, immunity and ameliorating adverse effect of oxidative stress during summer season. Its use has been found in improving both male and female reproductive health and production performance of bovines. Astaxanthin helps in hastening of sexual maturity, improving sperm quality, increasing fertility and better development of embryos. It is considered as one of the essential feed additive in combating the adverse effect of climate change on production performance of animals. This review mainly focuses on source, structure and use of astaxanthin as an antioxidant, anti-inflammatory, immune stimulator and for improving productive performance of bovines.

Keywords: Astaxanthin, Bovines, Heat stress, Immunity, Inflammation

Introduction

Antioxidants are “free-radical scavengers” that can evade slow damage to cells caused by free radicals, which are produced in body as a reaction to environmental and other stresses. In the animals, carotenoids are considered as part of the antioxidant defence system. Carotenoids are pigment that plays an essential role in mitigating oxidative processes. They are potent antioxidants that can scavenge mono-molecular oxygen and peroxy radicals. They influence cellular signalling and activate

redox sensitive controlling pathways (Stahl and Sies, 2005). High concentration of carotenoids is commonly found in plants, algae and microorganisms. Animals and humans do not synthesis these carotenoids, therefore its necessary to provide them in diet (Sandmann, 1994). Carotenoids acts by inhibiting chain reaction by taking out free radicals, as these carotenoids contain polyene chain and long double bonds they perform as potent antioxidant.

Astaxanthin is considered one of the strongest carotenoids which is capable of protecting cells, lipids and membranes lipoproteins from oxidative damage (Naguib, 2000). This review mainly focuses on source, structure and use of astaxanthin as an antioxidant, anti-inflammatory, immune stimulator and reproductive health of bovines.

Sources of astaxanthin (Ast)

Astaxanthin is predominantly found in organisms that are present in marine environment i.e. in microalgae, plankton, krill and seafood and also present in yeast, fungi, complex plants and the feathers of some birds (flamingos and quail) (Hussein et al. 2006). Astaxanthin was previously extracted from krill (*Euphausia superba*) (Takaichi et al. 2003) and recently from green algae (*Haematococcus pluvialis*) or red yeast (*Phaffia rhodozyma*) by various cultivation methods which help to obtain pure astaxanthin (Ambati, 2014) Astaxanthin obtained from algae are two times more effective as compared to synthetic astaxanthin (Guerin, 2001). *Haematococcus pluvialis* is a green microalga that builds up high levels of astaxanthine in stress environment like high salinity, deficiency of nitrogen content, high temperature and light (Choi et al. 2002). It is regarded as the foremost source of natural astaxanthin. Holtin et al. (2009) reported that *Haematococcus pluvialis* comprises approximately 4% of free form, 2% of diester type and 94% of monoester type.

Structure of astaxanthin

Astaxanthin consists of special molecular structure on each ionone ring, i.e. presence of hydroxyl and keto molecules, which are accountable for the potent antioxidant properties and superior position in the membrane (Liu and Osawa, 2007). Astaxanthin comprises conjugated double bonds, hydroxyl and keto groups

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(Figure 1). It has both lipophilic and hydrophilic properties (Higuera-Ciapara et al. 2006). Astaxanthin has two oxygenated groups on each ring structure that are responsible for its increased antioxidant characteristics (Guerin et al. 2003). In general lipid soluble antioxidants protect lipid soluble part of cells and water-soluble antioxidants protect the water-soluble portion of cells, but the unique structure of astaxanthin enables it to extend throughout cell membrane to play both roles and provides the cell membrane stability. Astaxanthin's polyene chain traps radicals in the cell membrane, while the astaxanthin terminal ring can scavenge radicals on the inner and outer parts of the cell membrane (Miki, 1991).

Naturally astaxanthin occurs in different forms (Table 1) esterified and free form and as isomers (Higuera-Ciapara et al. 2006). Synthetic astaxanthin is a molecule similar to that formed in living organisms and consists of a mixture of isomers 1:2:1 (3S, 3S), (3R, 3S), and (3R, 3R), respectively.

Mechanism of action of astaxanthin

Astaxanthin helps in neutralizing free radicals (nitrogen, sulphur, carbon and oxygen free radical) either by donating electrons to unpaired electrons or by quenching of unpaired electrons. Astaxanthin binds with free radical to form unreactive compound and help in quenching electron out of membrane (Figure 2) and resisting it from converting into pro-oxidant molecule.

Pharmacokinetics of Astaxanthin

Astaxanthin is carotenoid xanthophyll compound, is absorbed into body comparable to lipids and conveyed via lymphatic system (Figure 3). It get incorporated with lipoproteins and reaches tissues (Rao et al. 2013). Astaxanthin is a fat soluble compound, with enhanced absorption when supplemented along oils in diet. Absorption of astaxanthin also depends on associated dietary components. Bioavailability of astaxanthin in humans was improved when given with lipid-based diets, high levels of carotenes gets solubilized in the oil phase of the food matrix which can lead to better absorption (Olson, 2004; Rao et al. 2013). Lara et al. (2007) also stated that combination of astaxanthin and fish oil showed summative effect which contributed to improved immune response in wistar rats.

Biological Activity of Astaxanthin

Astaxanthin exhibited higher bioactivity than other antioxidants, since it could attach from inside to outside with cell membrane (Yuan et al. 2011). Astaxanthin is used as a nutrient in aquatic animals and as a pigmentation of broilers and egg yolk in the poultry industry (Cyanotech, 2000). Astaxanthin products are used as tablets, capsules, syrups, oils, soft gels, creams, biomass and granular powders for commercial applications in nutraceuticals. It acts as potent antioxidant, used as a dietary supplement to treat cardiovascular and neurodegenerative

Fig. 1 Chemical structure of Astaxanthin, Molecular formula - C₄₀H₅₂O₄ and Molar mass - 596.84 g/mol (Higuera-Ciapara et al. 2006)

Role of astaxanthin in molecular system (Biswal, 2014)

Quenching of singlet oxygen	Reduces free radical concentration
Removing of radicals	Prevent chain reactions
Inhibiting lipid peroxidation	Membrane structure is maintained
Regulation of gene expression	Improvement of immune system
Acts as COX-2 inhibitor	Reduces inflammation
Inhibits enzyme activity of iNOS	Reduces nitric oxide level
Reduces C-reactive protein (CRP),	Decreases systemic inflammation
Suppresses inflammatory gene expression	Subduing of NF-kappa Beta activation and I(kappa)B
Suppresses inflammatory agents	Prostaglandin E2, IL-10, TNFalfa
Anti-androgen effects	Inhibition of enzyme 5-a-reductase

Fig. 2: Presence of astaxanthin in cell membrane (Pashkow et al. 2008)

Table 1 Different forms of astaxanthin

Source of astaxanthin	Different forms	Reference
Antarctic krill (<i>Euphausia superba</i>)	3R, 32 R - occurs as the esterified form	(Foss et al. 1987)
Wild Atlantic salmon	3S, 32 S occurs as the free form	
<i>Haematococcus pluvialis</i>	(3S, 32 S) - occurs as the stereoisomer form	[Hussein et al. 2006]
<i>Xanthophyllomyces dendrorhous</i>	(3R, 32 R) - occurs as the stereoisomer form	

disorders, acts as anticancer agent, prevents diabetes, and stimulates immune system. It is used as a feed additive in combating adverse effect of heat stress in bovines (Table 2).

Astaxanthin as a potent antioxidant

Astaxanthin is known as “king of carotenoids” or “supervitamin E” (Pan et al. 2003). It is having both direct and indirect antioxidant actions. Directly scavenges free oxygen radicals to stop chain reactions involved in oxidative damage and indirectly activates enzymes of antioxidant defence system (Buesen et al. 2015). Astaxanthin’s ability to penetrate biological membranes with a defensive effect of lipid peroxidation within and outside of the cell membrane is one of its main features (Goto et al. 2001). Antioxidant strength of astaxanthin is 10 times more than zeaxanthin, lutein, canthaxanthin, β-carotene and 100 times greater than α-tocopherol and reported that many of carotenoid antioxidants like lycopene and zeaxanthin, or vitamins C and E under certain conditions become pro-oxidants but astaxanthin never turns into a pro-oxidant (Miki, 1991). Several in- vitro (Speranza et al. 2012) and in-vivo studies (Lee et al. 2011; Augusti et al. 2012) demonstrated antioxidant activity of astaxanthin.

Astaxanthin increases the activity of antioxidant enzymes (SOD, Catalase, GPx) considerably in astaxanthin treated LS-180 cell line (Hormozi et al. 2019) and in rats supplemented with astaxanthin orally (Rao et al. 2013). Yang (2011) reported that astaxanthin is capable of restoring the activity of non-enzymatic antioxidants such as GSH, vitamins C and E and other antioxidant

enzymes. Dietary supplementation of astaxanthin can provide antioxidant protection for cells and used in preventing atherosclerotic cardiovascular disease (Pashkow et al. 2008). It is considered as one of the potent antioxidant, as it increases the activity antioxidant enzymes (Choi et al. 2002). Increased expression of caspase 3 and Bax, which showed its anti-proliferative effects in the LS-180 cell line (Tang et al. 2018; Hormozi et al. 2019). Astaxanthin enhances liver function, upturns the potential for defence against oxidative stress and influences on mechanisms of biodefense significantly (Amar et al. 2001).

Astaxanthin has been used as dietary supplement in ameliorating adverse effect of oxidative stress in bovines in hot humid and hot dry season. Beneficial impact of astaxanthin supplementing in murrah buffaloes (Priyadarshini, 2017) and in Tharparkar and Karan Fries heifers (Kumar, 2018) helped to preventing adverse effects of summer stress and improved antioxidant defence system during the summer season. Higher levels of TAC in astaxanthin supplemented heifers helped in reducing the generation of singlet radicals during oxidative stress conditions. Decreased levels of TBARS and lipid peroxidation in treatment group indicated decreased levels of stress in heifers and potent antioxidant activity of astaxanthin in heifers (Kumar and Singh, 2020). Astaxanthin is able to ameliorate adverse effects of oxidative stress in lactating buffaloes by decreasing cortisol and other stress markers in supplemented group (Somagond et al. 2019). Dietary supplementation of astaxanthin during harsh climatic conditions makes animals more heat tolerant and resilient.

Table 2 Use of astaxanthin in bovines

Animal	Dosage	Effects of astaxanthin	Reference
Karan Fries bulls	0.25mg/kg BW/day/animal	Improved the semen quality during summer season	Soren et al. (2017) Soren and Singh (2018)
Pregnant dairy cows	1mg/kg body weight	Elevated corpus luteal (CL) function.	Kamada et al. (2017)
Murrah buffaloes (pre and postpartum Murrah buffaloes)	0.25mg/kg BW/day/animal	- Inhibited the level of inflammatory cytokines by blocking NF-κB activation - Improved the milk production performance of animal	Priyadarshini and Aggarwal, (2018)
Karan Fries and Tharparkar heifers	0.25mg/kg BW/day/animal	- Higher weight gain and early attainment of puberty during summer season - Improved the antioxidant status, immunity, growth rate and decreased rate of cell death	Kumar and Singh (2020).
Lactating Murrah Buffaloes	0.25mg/kg BW/day/animal	- Increased milk yield by 5.39% by astaxanthin supplementation alone and 15.6% by combination of astaxanthin + prill fat - Reduced physiological responses and stress indicators levels during summer stress	Somagond et al. (2019) and Somagond et al. (2020)

Anti-inflammatory and immunomodulatory action of astaxanthin

Astaxanthin is an effective antioxidant for terminating inflammation in biological systems. Several studies reported that astaxanthin mainly of marine origin showed its potent antioxidant and anti-inflammatory action (Fassett and Coombes, 2011). There was significant decrease in infection and gastric inflammatory conditions in *H. pylori*-infected mice when they are treated with algal cell extracts containing astaxanthin (Liu and Lee, 2003). Anti-inflammatory effects of astaxanthin shown through through inhibiting the expression genes like inducible NO synthase (iNOS), cyclooxygenase-2, tumor necrosis factor- α (TNF- α), and interleukin-1 β (IL-1 β) involved in pro-inflammatory and blocking of NF- κ B activation and reduced inflammatory mediators. Treatment with astaxanthin helps in reducing the secretion of pro-inflammatory cytokines from NF- κ B transcription factor by ROS induced production in H_2O_2 -stimulated mononuclear U937 cells (Speranza et al. 2012).

Astaxanthin is also involved in decreasing expression of Bcl2 genes and increases the expression of Caspase 3 and Bax genes, thereby, inhibiting the cells growth and proliferation and inducing apoptosis of cancer cells. So it was said that astaxanthin is also involved treating and prevention of cancers and strengthening the immune system in humans (Pashkow et al. 2008). Similarly Priyadarshini and Aggarwal (2018) reported dietary

Fig. 3 Pharmacokinetics of astaxanthin (Olson, 2004)

supplementation of astaxanthin in Murrah buffaloes prevented expression of genes involved in pro-inflammatory reactions, as a result of suppressing activation of NF κ B and decreased level of inflammatory mediators. Kumar and Singh (2019) revealed that impact of heat stress was ameliorated in Karan Fries and Tharparkar heifers through NF- κ B down regulation, which regulated the expression of genes involved in inflammatory reactions and quenching of free radicals. There were lower

cortisol levels in astaxanthin supplemented groups which showed heat stress alleviation effect during summer season.

In contrast to β -carotene and vitamin E, astaxanthin displayed greater immunomodulatory effects in mouse model (Jyonouchi, 1991). Studies in crustaceans, mainly on shrimp astaxanthin helped in improving immune response and increases tolerance to stress (Darachai et al. 1999). Dietary supplementation of astaxanthin increased antibody production and helped in restoring humoral immune response in older mice (Jyonouchi, 1991). Park et al. (2010) reported that astaxanthin acts as antiviral agent which is involved in enhancing natural killer cells activity, thereby destroying virus infected cells. Dietary supplementation of astaxanthin in Murrah buffaloes increased the amount of IgG levels, which could neutralise reactive oxygen species and protected immune cells from lipid peroxidation (Priyadarshini and Aggarwal, 2018).

Astaxanthin in reproductive health of animals

Effect on female reproduction

Despite the fact that astaxanthin is solemnly used for pigmentation and as potent antioxidant, it has many other uses relating to reproduction in fish, i.e., hastening of sexual maturity, improvement in fertilization, survival of eggs and better development of embryos (Putman, 1992). Negative effects of heat stress on in vitro embryo production in summer season can be ameliorated by dietary supplementation of astaxanthin. It helps in restoring reproductive performance of dairy cows exposed to heat stress through regulation oxygen free radicals and antioxidant enzymes in oocytes and cumulus-oocyte complexes (Ispada et al. 2018). Young et al. (1995) described that by dietary supplementation of astaxanthin derived from *Haematococcus pluvialis* was sufficient to elicit its antioxidant effect by detoxification of accumulated peroxides and improving ovary functions. Dietary supplementation of astaxanthin showed increase in corpora lutea (CL), implant sites and foetuses, thereby, decreasing abortion in Minks (Hansen et al. 2001). The addition of astaxanthin to the culture medium help in development of early bovine embryos in culture medium by ameliorating adverse effect of heat stress. In addition, astaxanthin promotes embryonic development during heat stress condition by altering expression genes related to stress (SHC1 and SOD2) (Namekawa et al. 2010). Addition of astaxanthin to maturation medium (0.5 ppm) improved growth and maturation of porcine oocyte and also increased the number of oocytes reaching metaphase II and considerably reduced apoptosis of oocytes exposed to H_2O_2 during in vitro maturation (IVM) (Do et al. 2015). Jang et al. (2010) revealed that addition of astaxanthin enhanced the growth of cultured embryos in bovine oviduct epithelial cells. Kuroki et al. (2013) also reported that astaxanthin supplementation during IVM enhanced mitochondrial membrane potential, which can help in rescuing oocyte from ROS generated due to heat induction.

Effect on male reproduction

In astaxanthin supplemented group of goldfish (*Carassius auratus*), higher sperm motility and fertilization rate was reported by Tizkar et al. (2015). Astaxanthin supplementation to human patient showed positive effect on sperm cells by decreasing the ROS and secretion of inhibin B, hence improving sperm quality and its function and increased conception rates or fertility (Comhaire, 2005). Concentrations of 2 μ M and 4 μ M of astaxanthin appeared to be successful dose to increase sperm potency during oxidative stress conditions (Salamon and Maxwell, 2000). Astaxanthin treatment in male mice greatly improved weight of testis and sperm morphological characteristics. Astaxanthin was able to restore sperm DNA damage because of generation of ROS in Cyclophosphamide (CP) induced toxicity. It also indicated germ cell protection of astaxanthin against chemo toxicity (Tripathi and Jena, 2008). Astaxanthin fed rats have shown a substantial reduction in ROS production in semen and improved male fertility (Mortazavi et al. 2014). Semen extender added with astaxanthin helped in improving the sperm quality and function of ram by reducing of malondialdehyde and ROS during storage (Fang et al. 2015) and bull (Farzan et al. 2014, Soren et al. 2017) semen at 5 °C for 72 hours. During production of miniature pig semen straw, adding of astaxanthin to frozen storage solutions, prevented damages during freeze-thawing process and maintained quality of sperms and improved fertility rate (Lee and Kim, 2018).

Kumar and Singh (2020) revealed that dietary supplementation of astaxanthin to Karan Fries and Sahiwal heifers helped in increasing level of growth hormone (GH), conservation of energy, increased growth performance and early attainment of puberty in supplemented group. Holstein bulls supplemented with astaxanthin enhanced the sperm progressive motility and reduced the lipid peroxidation rate in semen (Farzan et al. 2014). Addition of astaxanthin (0.25mg/kg body weight) revealed low level of antioxidant enzymes in semen, indicating positive effect on semen quality in Karan Fries bulls in heat stress conditions (Soren et al. 2017). Dietary Supplementation of astaxanthin in summer to crossbred (Karan Fries) bulls improved the quality of semen by improving antioxidant activity, which was indicated by decreased malondialdehyde concentration and higher expression of mitochondrial gene in spermatozoa like, mitochondrial transcriptions factor A (TFAM), citrate synthases (CS) and succinate dehydrogenase (SDH). Supplementation with astaxanthin also enhanced semen quality parameters (volume, motility, concentration, and acrosome integrity) and reduced major abnormalities in semen over non supplemented bulls. Therefore, astaxanthin supplementation can be used as one of strategies in improving semen quality of crossbred bulls and amelioration of adverse effect of heat stress was suggested by Soren and Singh (2018).

Astaxanthin which acts as a potent antioxidant helps in amelioration of adverse effect of heat stress and improving productive performance of animals. Kumar and Singh (2020) reported astaxanthin supplementation in diets of heifers helped in improvement of growth, immunity, which subsequently leads to early attainment of puberty and increased productive life of cattle. Astaxanthin supplementation along with prill fat was able to maintain body condition and health status of buffaloes by mitigating adverse effect during summer season (Somagond et al. 2020). Priyadarshni (2017) revealed that astaxanthin supplementation enhanced immunity and lowered stress marker levels in Murrah buffaloes and improved reproductive health and milk yield by 7% during heat stress condition. There was 5.39% and 15.6% increase in milk yield in astaxanthin and astaxanthin in combination with prill fat fed groups of buffaloes over control group, respectively during summer season (Somagond et al. 2019).

Conclusion

Astaxanthin as a feed additive helps in amelioration of adverse effects of heat stress, acts as anti-inflammatory, improves immunity, reduces stress levels in bovines. Therefore, astaxanthin can be used as an important feed additive to combat the adverse effect of climate change/ stress and for improving production performance of animals during adverse climatic conditions.

References

- Amar EC, Kiron V, Satoh S, Watanabe T (2001) Influence of various dietary synthetic carotenoids on bio defence mechanisms in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac Res* 32: 162-173
- Ambati RR, Phang SM, Ravi S, Aswathanarayana, RG (2014) Astaxanthin: sources, extraction, stability, biological activities and its commercial applications - a review. *Mar Drugs* 12: 128-152
- Augusti PR, Quattrin A, Somacal S, Conterato GM, Sobieski R, Ruviano AR, and Emanuelli T (2012) Astaxanthin prevents changes in the activities of thioredoxin reductase and paraoxonase in hypercholesterolemic rabbits. *J Clin Biochem Nutr* 51: 42-49
- Biswal S (2014) Oxidative stress and astaxanthin: The novel supernutrient carotenoid. *Int J Res Health Allied Sci* 3: 147
- Buesen R, Schulte S, Strauss V, Treumann S, Becker M, Groters S, Van Ravenzwaay B (2015) Safety assessment of [3S, 3Z]-astaxanthin-Subchronic toxicity study in rats. *Food Chem Toxicol* 81:129-136
- Choi YE, Yun YS, Park JM (2002) Evaluation of factors promoting astaxanthin production by a unicellular green alga, *Haematococcus pluvialis*, with fractional factorial design. *Biotechnol Prog* 18: 1170-1175
- Comhaire FH, Garem YE, Mahmoud A, Eertmans F, Schoonjans FRANK (2005) Combined conventional/antioxidant "Astaxanthin" treatment for male infertility: a double blind, randomized trial. *Asian J Androl* 7: 257-262
- Cyanotech 2000 NatuRose®. Available at www.cyanotech.com/html/asta-natu/naturrose.html [accessed 12 December 2007]
- Darachai J, Piyatiratitivorakul S, Menasveta P (1999) Effect of astaxanthin on growth and survival of *Penaeus monodon* larvae. In Proceedings of the 37th Kasetsart University Annual Conference 36-41
- Do LTK, Luu VV, Morita Y, Taniguchi M, Nii M, Peter AT, Otoi T (2015) Astaxanthin present in the maturation medium reduces negative effects of heat shock on the developmental competence of porcine oocytes. *Reprod Biol* 15: 86-93
- Fang YI, Zhong R, Chen L, Feng C, Sun H, Zhou D (2015) Effects of astaxanthin supplementation on the sperm quality and antioxidant capacity of ram semen during liquid storage. *Small Rumin Res* 130: 178-182
- Farzan M, Chamani M, Varnaseri H (2014) The antioxidant effect of astaxanthin on quantitative and qualitative parameters of bull sperm. *Indian Journal of Fundamental and Applied Life Sciences* 4: 425-430
- Fassett RG, Coombes JS (2011) Astaxanthin: a potential therapeutic agent in cardiovascular disease. *Mar Drugs* 9(3): 447-465
- Foss P, Renström B, Liaaen-Jensen S (1987) Natural occurrence of enantiomeric and Meso astaxanthin 7"-crustaceans including zooplankton. *Comp Biochem Physiol B* 86: 313-314
- Goto S, Kogure K, Abe K, Kimata Y, Kitahama K, Yamashita E, Terada H (2001) Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent antiperoxidative activity of the carotenoid astaxanthin. *Biochim Biophys Acta Biomembr* 1512: 251-258
- Guerin M (2001) Pigmentation of red seabream with natural astaxanthin derived from the alga *Haematococcus pluvialis*: comparison with synthetic astaxanthin. In *Aquaculture 2001 conference*, Lake Buena Vista, Florida, January 2001. World Aquaculture Society
- Guerin M, Huntley ME, Olaizola M (2003) *Haematococcus* astaxanthin: applications for human health and nutrition. *Trends Biotechnol* 21: 210-216
- Hansen KB, Tauson AH, Inborr J (2001) Effect of supplementation with the antioxidant astaxanthin on reproduction, pre-weaning growth performance of kits and daily milk intake in mink. *J Reprod Fertil Suppl* 57: 331-334
- Higuera-Ciapara I, Felix-Valenzuela L, Goycoolea FM (2006) Astaxanthin: a review of its chemistry and applications. *Crit Rev Food Sci Nutr* 46: 185-196
- Holtin K, Kuehnle M, Rehbein J, Schuler P, Nicholson G, Albert K (2009) Determination of astaxanthin and astaxanthin esters in the microalgae *Haematococcus pluvialis* by LC-(APCI) MS and characterization of predominant carotenoid isomers by NMR spectroscopy. *Anal Bioanal Chem* 395: 1613
- Hormozi M, Ghoreishi S, Baharvand P (2019) Astaxanthin induces apoptosis and increases activity of antioxidant enzymes in LS-180 cells. *Artif Cells Nanomed Biotechnol* 47: 891-895
- Hussein G, Sankawa U, Goto H, Matsumoto K, Watanabe H (2006) Astaxanthin, a carotenoid with potential in human health and nutrition. *J. Nat. Prod* 69: 443-449
- Ispada J, Rodrigues TA, Risolia PHB, Lima RS, Gonçalves DR, Rettori D, Paula-Lopes FF (2018) Astaxanthin counteracts the effects of heat shock on the maturation of bovine oocytes. *Reprod Fert Dev* 30: 1169-1179
- Jang HY, Ji S J Kim, YH Lee, HY Shin, JS, Cheong HT, Yang BK (2010) Antioxidative effects of astaxanthin against nitric oxide induced oxidative stress on cell viability and gene expression in bovine oviduct epithelial cell and the developmental competence of bovine IVM/IVF embryos. *Reprod Domest Anim* 45: 967-974
- Jyonouchi H, Hill RJ, Tomita Y, Good RA (1991) Studies of immunomodulating actions of carotenoids. I. Effects of β carotene and astaxanthin on murine lymphocyte functions and cell surface marker expression in in vitro culture system. *Nutr Cancer* 16: 93-105
- Kamada H, Akagi S, Watanabe S (2017) Astaxanthin increases progesterone production in cultured bovine luteal cells. *J Vet Med Sci* 17-0044
- Kumar S (2018) Influence of astaxanthin supplementation on growth performance, immunity and antioxidant status in heifers during

- summer season (Doctoral dissertation, National Dairy Research Institute, Karnal)
- Kumar S, Singh SV (2019) Inhibition of NF- κ B signaling pathway by astaxanthin supplementation for prevention of heat stress-induced inflammatory changes and apoptosis in Karan Fries heifers. *Trop Anim Health Pro* 51: 1125-1134
- Kumar S, Singh SV (2020) Influence of astaxanthin supplementation on attainment of puberty and lipid peroxidation in Sahiwal and Karan Fries (Holstein $\times$ Tharparkar) heifers during summer season. *Biol Rhythm Res* 51: 15-28
- Kuroki T, Ikeda S, Okada T, Maoka T, Kitamura A, Sugimoto M, Kume S (2013) Astaxanthin ameliorates heat stress-induced impairment of blastocyst development *In Vitro*:—astaxanthin colocalization with and action on mitochondria. *J Assist Reprod Gen* 30: 623-631
- Lara JJ, Economou M, Wallace AM, Rumley A, Lowe G, Slater C, Lean ME (2007) Benefits of salmon eating on traditional and novel vascular risk factors in young, non-obese healthy subjects. *Atherosclerosis* 193: 213-221
- Lee DH, Kim CS, Lee YJ (2011) Astaxanthin protects against MPTP/MPP⁺-induced mitochondrial dysfunction and ROS production in vivo and in vitro. *Food Chem. Toxicol* 49: 271-280
- Lee E, Kim D (2018) Effects of astaxanthin on miniature pig sperm cryopreservation. *Biomed Res Int.* doi.org/10.1111/and.13389.
- Liu BH, Lee YK (2003). Effect of total secondary carotenoids extracts from *Chlorococcum* sp. on *Helicobacter pylori*-infected BALB/c mice. *Int Immunopharmacol* 3: 979-986.
- Liu X, Osawa T (2007) Cis astaxanthin and especially 9-cis astaxanthin exhibits a higher antioxidant activity in vitro compared to the all-trans isomer. *Biochem Biophys Res Commun* 357: 187-193
- McNulty H, Jacob RF, Mason RP (2008) Biologic activity of carotenoids related to distinct membrane physicochemical interactions. *Am J Cardiol* 101: S20-S29
- Miki W (1991) Biological functions and activities of animal carotenoids. *Pure Appl Chem* 63: 141-146
- Mortazavi M, Salehi I, Alizadeh Z, Vahabian M and Roushandeh AM (2014) Protective effects of antioxidants on sperm parameters and seminiferous tubules epithelium in high fat-fed rats. *J Reprod Infertil* 15: 22
- Naguib YM (2000) Antioxidant activities of astaxanthin and related carotenoids. *J Agric Food Chem* 48: 1150-1154
- Namekawa T, Ikeda S, Sugimoto M, Kume S (2010) Effects of astaxanthin containing oil on development and stress related gene expression of bovine embryos exposed to heat stress. *Reprod Domest Anim* 45(6): e387-e391
- Olson J A (2004) Carotenoids: absorption, transport, and metabolism of carotenoids in humans. *Pure Appl Chem* 66: 1011-1016
- Pan CH, Chien YH, Hunter B (2003) The resistance to ammonia stress of *Penaeus monodon*, *Fabricius juvenile* fed diets supplemented with astaxanthin. *J Exp Mar Biol Ecol* 297: 107-118
- Park JS, Chyun JH, Kim YK, Line LL, Chew BP (2010) Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. *Nutr. Metab* 7: 18
- Pashkow FJ, Watumull DG, Campbell CL (2008) Astaxanthin: a novel potential treatment for oxidative stress and inflammation in cardiovascular disease. *Am J Cardiol* 101: S8-S68
- Priyadarshini L (2017) Immunity, antioxidant, energy status and production performance in pre and post-partum Murrah buffaloes during summer and winter seasons (Doctoral dissertation, National Dairy Research Institute; Karnal)
- Priyadarshini L, Aggarwal A (2018) Astaxanthin inhibits cytokines production and inflammatory gene expression by suppressing IkB kinase-dependent nuclear factor κ B activation in pre and postpartum Murrah buffaloes during different seasons. *Vet World* 11: 782
- Putman M (1992) A review of the nature, function, variability, and supply of pigments in salmonid fish. *EAS Special Publication*
- Rao AR, Sindhuja HN, Dharmesh SM, Sankar KU, Sarada R, Ravishankar GA (2013) Effective inhibition of skin cancer, tyrosinase, and antioxidative properties by astaxanthin and astaxanthin esters from the green alga *Haematococcus pluvialis*. *J Agric Food Chem* 61: 3842-3851
- Salamon S, Maxwell WMC (2000) Storage of ram semen. *Anim. Reprod Sci* 62: 77-111
- Sandmann G (1994) Carotenoid biosynthesis in microorganisms and plants. In *EJB Reviews*. Springer, Berlin, Heidelberg 129-146
- Somagond YM, Singh S, Deshpande A, Sheoran P, Chahal VP (2020) Physiological responses, energy metabolites and prolactin levels of buffaloes supplemented with dietary astaxanthin, prill fat and their combination during heat stress. *Indian J Anim Sci* 90: 55-60
- Somagond YM, Singh SV, Deshpande A (2019) Effect of dietary supplementation of astaxanthin, prill fat and combination on stress indicators, milk yield and composition during heat stress in buffaloes. *Biol Rhythm Res* 1-11. doi.org/10.1080/09291016.2019.1658426
- Soren S, Singh SV (2018) Semen quality, lipid peroxidation and expression of mitochondrial gene in ejaculated sperm of Karan Fries (Tharparkar $\times$ Holstein Friesian) bulls supplemented with astaxanthin. *Indian J Anim Sci* 88: 1346-1352
- Soren S, Singh SV, Kumar S (2017) Effect of astaxanthin supplementation on semen (Karan Fries Bulls) storage at 5 C. *Int J Curr Microbiol Appl Sci* 6: 23-28
- Speranza, L, Pesce M, Patruno A, Franceschelli S, Lutiis MAD, Grilli A, Felaco M (2012) Astaxanthin treatment reduced oxidative induced pro-inflammatory cytokines secretion in U937: SHP-1 as a novel biological target. *Mar Drugs* 10: 890-899
- Stahl W, Sies H (2005) Bioactivity and protective effects of natural carotenoids. *Biochim Biophys Acta Mol Basis Dis* 1740: 101-107
- Takaichi S, Matsui K, Nakamura M, Muramatsu M, Hanada S (2003) Fatty acids of astaxanthin esters in krill determined by mild mass spectrometry. *Comp Biochem Physiol B Biochem Mol Biol* 136: 317-322
- Tang X, Liu B, Wang X, Yu Q, Fang R (2018) Epidermal growth factor, through alleviating oxidative stress, protect IPEC-J2 cells from lipopolysaccharides-induced apoptosis. *Int J Mol Sci* 19: 848
- Tizkar B, Kazemi R, Alipour A, Seidavi A, Naseralavi G, Ponce-Palafox JT (2015) Effects of dietary supplementation with astaxanthin and β -carotene on the semen quality of goldfish (*Carassius auratus*). *Theriogenology* 84: 1111-1117
- Tripathi DN, Jena GB (2008) Astaxanthin inhibits cytotoxic and genotoxic effects of cyclophosphamide in mice germ cells. *Toxicology* 248: 96-103
- Yang Y, Seo JM, Nguyen A, Pham TX, Park HJ, Park Y, Lee J (2011) Astaxanthin-rich extract from the green alga *Haematococcus pluvialis* lowers plasma lipid concentrations and enhances antioxidant defense in apolipoprotein E knockout mice. *J Nutr* 141: 1611-1617
- Young FM, Luderer WB, Rodgers RJ (1995) The antioxidant β -carotene prevents covalent cross-linking between cholesterol side-chain cleavage cytochrome P450 and its electron donor, adrenodoxin, in bovine luteal cells. *Mol Cell Endocrinol* 109: 113-118
- Yuan JP, Peng J, Yin K, Wang JH (2011) Potential health promoting effects of astaxanthin: A high value carotenoid mostly from microalgae. *Mol Nutr Food Res* 55: 150-165