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Potential Immuno-stimulatory Nutrients for the Equine Athlete

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Introduction

Exercise is widely recognized as a stressor, causing neuroendocrine and hormonal changes that can mediate alterations in immune function, thereby influencing susceptibility to disease. Viral infections result in lost training time and decreased earnings over the course of the horse's athletic career. Although a reduction in training load could help alleviate disease risk, this is often impractical when preparing horses for competition. Hence, strategies to mitigate exercise-induced immunosuppression would be of use to the horse industry. Nutrition plays a supportive role in immunity, and some nutrients appear to possess immuno-stimulatory or modulatory effects when supplemented to the diet. Research in humans suggests that nutrient supplementation may provide a means of modifying changes in immune function following strenuous or prolonged exercise. Study on the interactions between nutrition and immune response to training and competition are currently lacking in the equine athlete. As a point of discussion, this paper will highlight immuno-modulating nutrients that have shown promise in human athletes and could provide direction for future research in the performance horse.

Overview of the Immune System

The immune system is fascinating, but can be somewhat daunting in its complexity. Nonetheless, knowledge of immune function is important for understanding how nutrition can potentially be used to modify immune response. The following discussion provides an overview of some of the key features of the immune system, with focus primarily on aspects that may be discussed in this paper. For more detailed descriptions, the reader is encouraged to seek the many excellent reviews and books on the topic (e.g., Calder 2007; Tizard 2004).

The immune system has two functional divisions: 1) the innate (also termed non-specific or natural) and, 2) the acquired (also termed specific or adaptive). Both components of immunity involve a variety of cell types, mediators, and chemical agents (Figure 1), which act in a coordinated fashion to eliminate infectious agents (bacteria, viruses, fungi, parasites) and tumor cells, as well as respond to injury and trauma.

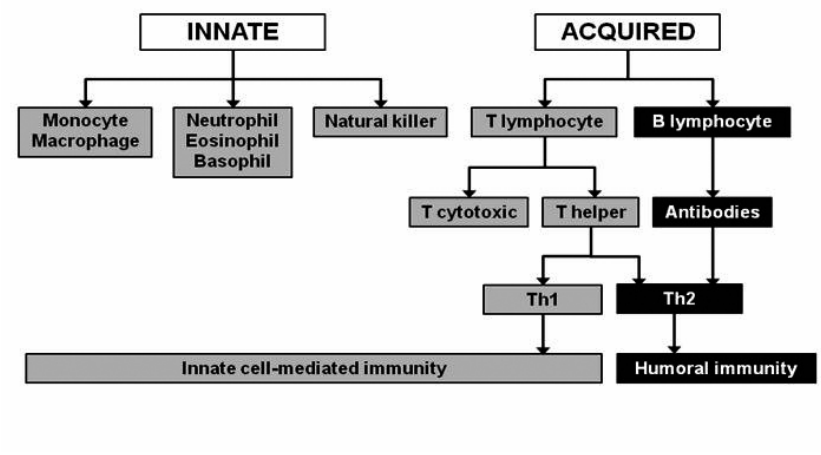

Figure 1: Divisions of the immune system (adapted from Smith 2003).

Innate immunity is the first line of defence against infectious agents. It exists before exposure to pathogens and is concerned with preventing the entry of infectious agents and with rapid elimination if they do enter the body. Innate

immunity has no memory and is therefore not influenced by prior exposure to an organism. The innate immune system includes physical barriers (e.g., skin, stomach acid, mucosal membranes), soluble factors (e.g., complement), phagocytic cells (e.g., neutrophils, monocytes/macrophages) and natural killer cells (Figure 1). Phagocytes engulf bacteria (referred to as phagocytosis) and destroy them by complement or by the production of toxic chemicals, such as superoxide radicals and hydrogen peroxide (referred to as oxidative burst). By comparison, natural killer (NK) cells destroy infected cells via the release of cytotoxic proteins.

The acquired immune response involves lymphocytes (Figure 1). It is highly specific because each lymphocyte carries surface receptors for a single antigen. The acquired immune response becomes active over several days after the initial activation, but it also persists for some time after the removal of the initiating antigen. This persistence gives rise to immunological memory, which is the basis for a stronger, more effective immune response upon reexposure to an antigen (i.e., reinfection with the same pathogen).

There are two main types of lymphocytes: B lymphocytes (B-cells) and T lymphocytes (T-cells). B-cells are characterized by their ability to produce antibodies (immunoglobulins, Ig), which are specific for an individual antigen. Antibodies can neutralize microorganisms by binding to them and preventing their attachment to host cells. B-cells can also activate complement proteins in plasma, which in turn promote the destruction of bacteria by phagocytic cells from the innate immune system. Immunity involving antibodies–often referred to as humoral immunity–deals with extracellular pathogens. Intracellular pathogens (e.g., viruses, some bacteria) will escape humoral immunity and are instead dealt with by cell-mediated (or cellular) immunity. Cell-mediated immunity is conferred by T-cells. T-cells express antigen-specific receptors on their surface; however, unlike B-cells, they are only able to recognize those antigens that are “presented” to them on the surface of an infected cell (i.e., an antigen-presenting cell). This is the distinguishing feature between humoral and cell-mediated immunity (Figure 1).

T-cells can be divided into many different subpopulations, based on the antigen receptors they possess, the accessory molecules supporting their activity, and ultimately their function. In general, cytotoxic T-cells (Tc) eliminate intracellular pathogens by destroying the infected cell, similar to NK cells. In contrast, helper T-cells (Th) respond to pathogens by secreting cytokines (described more below), which have several effects, including the recruitment of other immune cells to the site of challenge. Th cells can be divided into two distinct subsets, referred to as helper T1-cells (Th1) and T2-cells (Th2). Th1-cells generate resistance to intracellular organisms and are associated with cell-mediated immunity. In contrast, Th2-cells target extra cellular pathogens and are associated with humoral immunity (Figure 1).

Communication within the acquired immune system and between the innate and acquired systems is brought about by direct cell-to-cell contact involving cell surface proteins (e.g., adhesion molecules) and by the production of chemical messengers. Chief among these chemical messengers are proteins called cytokines, which bind to specific receptors on the cell surface and induce changes in growth, development, or activity of the cell.

Currently, more than 100 cytokines have been identified. Cytokines are generally grouped into the following families: interleukins (IL-1 to 30); 2); interferons (IFNα, β, γ, etc.); tumor necrosis factors (TNFα, β, etc); haematopoietic growth factors, including various colony simulating factors; and chemotactic factors (termed chemokines).

Cytokines are synthesized by a variety of immune cells, especially macrophages and lymphocytes, but can also be produced by other cells such as endothelial cells and muscle. Some cytokines, such as IL-1, are synthesized by nearly all nucleated cell types, while at the other end of the spectrum, IL-2 and INFγ are lymphocyte (Th1) specific. Cytokines are not produced in advance and stored, but rather rapidly synthesized or suppressed following cellular activation. There is some redundancy among cytokines in that many have similar effects. In addition, some cytokines act synergistically or antagonistically with other cytokines. Some of the key cytokines and their actions are summarized in Table 1.

Table 1: Some key cytokines and their roles in immune response.

Cytokine	Predominantly Pro- or Anti-Inflammatory	Action(s)
TNFα	Pro	• Potent inflammatory molecule • Activates adhesion molecules, procoagulants and induces acute phase proteins • Activates most leukocytes & vascular endothelium
TNFα-sr	Anti	• TNFα soluble receptor; inactivates TNFα
IL-1β	Pro	• Co-stimulator of Th2 cells • Key role in fever, hematopoiesis, appetite control • Acts on most leukocytes, endothelial cells, hepatocytes
IL-1ra	Anti	• IL-1 receptor antagonist; blocks activity of IL-1
IL-2	Pro	• Promotes proliferation of antigen-specific T-cells
IL-4	Both	• Promotes differentiation and activity of Th2-cells (and thus inhibits differentiation and activity of Th1-cells) • Promotes generation of antigen-specific IgE by B-cells
IL-5	Pro	• Promotes activation of eosinophils
IL-6	Both	• Stimulates acute phase response • Promotes B-cell differentiation and maturation into plasma cells • Co-stimulator of Th2-cell activity
IL-6sr	Anti	• IL-6 soluble receptor; inactivates IL-6
IL-10	Anti	• Targets Th1, B, NK and mast cells and macrophages • Inhibits synthesis of Th1 cytokines (IL-2, IFNγ, TNFα) • Suppresses secretion of IL-1, IL-6, TNFα by macrophages
INFγ	Pro	• Promotes differentiation and activity of Th1-cells (and thus inhibits differentiation and activity of Th2-cells) • Activates cells involved in elimination of bacteria, viruses, fungi and tumor cells (e.g., monocytes, macrophages, cytotoxic T cells, NK cells)

Cytokines are important in directing local and coordinating whole body immune responses to infection or injury. TNFα, IL-1β, and IL-6 produced by monocytes and macrophages are particularly important in this process. These cytokines serve to regulate the response to infection and injury by activating neutrophils, monocytes, and macrophages to initiate bacterial or tumor cell killing, stimulate T- and B-cell proliferation, and initiate the production of other pro-inflammatory cytokines. Thus, these cytokines are mediators of both innate and acquired immunity and are an important link between them. In addition, these cytokines mediate the systemic effects of inflammation such as fever, weight loss, and acute-phase protein synthesis in the liver. Inflammation is the body's immediate response to infection or injury and is an integral part of the innate immune response. Production of appropriate amounts of TNFα, IL-1β, and IL-6 is an important response to infection; however, inappropriate production or overproduction can be dangerous, and these cytokines (particularly TNFα) are implicated in causing some of the pathological responses that occur in acute and chronic inflammatory conditions (Calder et al. 2002).

There is a wide range of methodologies available with which to assess the status and functional capacity of the immune system. The activities of the separate components of the immune system are most frequently measured by studying that component under controlled *ex vivo* (i.e., outside the body) conditions. For example, the functional response of various immune cells can be assessed by isolating them from the blood or relevant tissues (e.g., thymus, lymph nodes, liver, gut, lungs, and bone marrow) and stimulating them with an antigen or mitogen. In this manner, it is possible to assess the chemotactic ability (i.e., ability to travel towards a particular stimuli) of neutrophils and monocytes, as well as their ability to undergo phagocytosis and oxidative burst to eliminate pathogens. Similarly, the ability of T cells and B cells to increase in number (i.e., lymphocyte proliferation) in response to a stimulus can be measured. Cytokine production (or mRNA expression) by stimulated T cells and monocytes can also be measured. It is also possible to study a coordinated immune response *in vivo*, usually to a controlled challenge, such as antibody response to a vaccination or the size of swelling (induration) in response to an intradermal application of an antigen. However, it must be remembered that there is no single marker of either the status of the immune system or its functional capacity. Therefore, several different measurements must be obtained to more accurately characterize the impact of a given treatment (e.g., nutrition) on immune function.

Impact of Exercise on the Immune System

Exercise is widely recognized as a stressor that can influence the immune system and disease susceptibility. It is beyond the scope of this paper to provide a comprehensive review on the effects of exercise on immune function; rather, a general overview will be provided. The reader is directed towards a bounty of reviews that exist on this subject in humans (e.g., Brolinson and Elliott 2007; Gleeson 2007; Nieman and Pedersen 1999; Pedersen and Hoffman-Goetz 2000; Smith 2003) and horses (Art and Lekeux 2005; Trogden Hines et al. 1996).

The interaction between exercise and immune function is complex and, as a result, the effects vary depending on the duration and intensity of exercise, as well as the subject's current level of fitness. In general, regular moderate intensity exercise is associated with beneficial effects on host defence mechanisms. In contrast, acute bouts of high intensity activity or prolonged exercise can result in immunosuppression, primarily of the innate immune system. A study conducted in horses by Raidal et al. (2000) is a great illustration of this phenomenon. Untrained horses undergoing moderate intensity exercise (30-40% maximal oxygen consumption, VO_{2MAX}) exhibited improved neutrophil phagocytosis and oxidative burst activity compared to sedentary horses. However, high intensity exercise (run to fatigue at 115% VO_{2MAX}) resulted in a transient decrease in neutrophil phagocytosis and a more prolonged decrease (through 6 hours post-exercise) in oxidative burst activity in the same untrained horses. After the horses underwent a 10-week endurance training program, the suppressive effects noted in response to the first high intensity exercise test were no longer apparent in response to a second high intensity exercise test. In contrast, after a further 6 weeks of training at high intensity, neutrophil phagocytosis and oxidative burst activity were decreased in response to a third high intensity exercise test.

A summary of the impacts of strenuous exercise on the immune system in humans and laboratory animals is presented in Table 2. Although less well studied, similar effects appear to occur in horses (Adamson and Slocombe 1995; Buschmann and Baumann 1991; Colahan et al. 2002; Donovan et al. 2007a, b; Escribano et al. 2005; Jensen-Waern et al. 1999; Keadle et al. 1993; Nesse et al. 2002; Raidal et al. 2000; Wong et al. 1992).

Table 2: Summary of the effects of strenuous exercise on the immune system.

	During Exercise	After Exercise
Leukocyte Numbers		
Neutrophil count	↑	↑↑
Monocyte count		↑
Th1-cell count	↑	↓
Th2-cell count	↑	0
B-cell count	↑	↓
NK-cell count	↑	↓
Leukocyte Activity		
Lymphocyte proliferative response to mitogens	↓	↓
Neutrophil phagocytosis and oxidative burst	↓	↓
NK cell activity	↑	↓
Lymphokine activated killer cell activity	↑	↓
Antibody response *in vitro*	↓	↓
Antibody response *in vivo*		0
Plasma Cytokine Concentrations		
TNFα	↑	↑
IL-1	↑	↑
IL-6	↑↑	↑
IL-1ra	↑↑	↑
IL-10	↑	↑
TNFα-sr	↑	↑

A substantial increase in the number of circulating immune cells occurs during exercise (primarily neutrophils and lymphocytes), the magnitude of which is related to both the intensity and duration of exercise (Table 2). Changes in the proportion of immune cells in circulation reflect the recruitment of cells to the vascular compartment during exercise and the redistribution of cells upon cessation of exercise (Pedersen and Hoffman-Goetz 2000). Although the activity of a subpopulation of immune cells can be influenced by their relative proportion in plasma, the activity of most immune cells appears to decrease after strenuous exercise when evaluated on a per cell basis (Table 2). There are also increases in the plasma concentrations of various substances that are known to influence immune cell function, including the more potent pro-inflammatory cytokines, TNFα and IF-1β, as well as increased levels of anti-inflammatory cytokines, such as IL-6, TNFα-sr, IF-1ra, and IL-10 (Table 2). Interestingly, the large increase in IL-6 originates predominantly from muscle production, rather than from immune cells (Pedersen et al. 2007). Hormonal changes also occur in response to exercise, including increased levels of epinephrine, cortisol, growth hormone and prolactin, which are known to have immunomodulatory effects (Pedersen and Hoffman-Goetz 2000). Muscle-derived IL-6 appears to be at least partly responsible for the elevated secretion of cortisol (Gleeson 2007). Ultimately, an acute bout of high intensity exercise or prolonged strenuous activity is accompanied by responses that are remarkably similar in many respects to those induced by infection, sepsis or trauma (Northoff et al. 1998).

Recently, the exercised-induced alterations in immunity have been attributed to the influence of exercise on the balance of Th1/Th2 cells and the cytokines they produce (Gleeson 2007; Smith 2003). Strenuous exercise decreases the percentage of Th1 cells in circulation, whereas the percentage of Th2 cells does not change (Table 2). This is important, because the type of cytokines released by activated Th cells will largely dictate whether humoral or cell-mediated immunity will dominate. Both cortisol and epinephrine suppress cytokine production from Th1 cells, whereas IL-6 directly stimulates Th2 cell cytokine production (Gleeson 2007) (Table 1). Because Th1 cells promote cell-mediated immunity, which primarily provides protection against intracellular pathogens (e.g., viruses), it has been suggested that

exercise, possibly working through muscle-derived IL-6, may decrease virus protection in the host by shifting the balance towards Th2 cell cytokine production (Smith 2003). Although this could increase disease susceptibility, the shift towards Th2-cell dominance is also beneficial, because it can suppress the ability of the immune system to induce tissue damage and inflammation.

The changes in circulating immune cell numbers and functional activity following strenuous exercise normally return to pre-exercise values within 2 to 72 hours, depending on the parameter measured. This period of immunosuppression has been suggested to provide an "open window" for infection, representing the most vulnerable time period for an athlete in terms of their susceptibility to disease (Gleeson 2007). However, despite these changes, few studies have been able to demonstrate a direct association between any specific measure of exercise-induced impaired immune function and increased incidence of clinically confirmed infection. That is to say, subjects that show the most extreme post-exercise immuno-suppression have not necessarily been shown to be those that contract an infection during the ensuing 1 to 2 weeks. Nonetheless, exercise training has been associated with increased susceptibility to viral infection in horses. Ponies undergoing a 5-day strenuous exercise program had decreased *in vitro* cell-mediated immune responses to influenza virus and increased susceptibility to influenza disease following an *in vivo* challenge exposure to the virus compared with non-exercised control ponies (Folsom et al. 2001).

Finally, it is important to recognize that although several aspects of both innate and adaptive immunity are depressed during chronic heavy training, athletes are not clinically immune deficient (Nieman and Pedersen 1999). In other words, exercise-induced immune dysfunction does not necessarily put the performance horse in danger of serious illness, but it could be sufficient to increase the risk of contracting common infections. And when combined with environmental stressors that typically accompany a heavily campaigned performance horse (e.g., frequent transport over long distances, concentrated housing at show and racetrack facilities) exposure to airborne pathogens and disease susceptibility can be further elevated (Art and Lekeux 2005). Even minor infections can result in a drop in exercise performance and the ability to sustain heavy training. Therefore, strategies to mitigate risk of contracting an infection would be of great use to most performance horses. The use of nutrition as an immunomodulator is one such strategy.

Application of Immunonutrition to the Performance Horse

Nutrition plays a supportive role in immunity and host defence (see Calder et al. 2002). Table 3 summarizes some of the key nutrients involved in immune function. For example, various amino acids (i.e., glutamine, arginine, methionine and cysteine) and minerals (i.e., zinc and selenium) are critical to the formation, proliferation and functional activity of immune cells and other components of the immune system. Vitamin A (as well as lycopene and lutein) and vitamins E and C are also important in immune cell function, as well as mitigation of reactive oxygen species generated by the highly metabolic immune cells participating in host defence. In addition, polyunsaturated fatty acids, acting via their conversion to eicosanoids, are responsible for directing many activities associated with inflammation and immune response. Deficiencies or imbalances of these nutrients generally result in compromised immune function and decreased disease resistance. Therefore, a balanced diet is critical to mount an appropriate immune response to infection or trauma.

Based on the role of nutrition in immunity, it has been proposed that the transient immuno-suppression seen in response to intensive training may be alleviated with strategic nutrient supplementation (Pedersen and Hoffman-Goetz 2000; Gleeson 2007). This area of study has not yet received a lot of attention in the performance horse, but has been gaining momentum in the human athlete. Therefore, the remainder of this paper will highlight nutrients that have shown the most promise in modifying immune response in human athletes, and which could potentially be investigated further in the horse.

Table 3: Immunomodulating nutrients

Nutrient	Effects on the Immune System
Glutamine	• Important energy source for immune cells • Nitrogen donor for purine/pyrimidine synthesis (RNA/DNA) • T-cell proliferation, B-cell differentiation, macrophage function, cytokine production • Component of glutathione (antioxidant defense)
Arginine	• Needed for normal growth and proliferation of lymphocytes • Increases macrophage and NK cell cytoxicity • Wound healing
Cysteine (or methionine)	• Component of glutathione (antioxidant defense)
Zinc	• Plays key role in immune cell signaling, activation, gene expression, protein synthesis and apoptosis • Crucial for normal development of immune cells • Maintain activity of neutrophils, monocytes/macrophages, NK cells, B-cells, and T-cells
Selenium	• Immune cell integrity (via glutathione peroxidase) • Maintenance of receptor protein structure (via thioredoxin reductase) • B-cell differentiation and antibody production • Expression of some cytokines
Vitamin A	• Maintenance of mucosal surfaces (first line of defense) • Generation of antibody responses • Immune cell proliferation & function (via gene transcription)
Lycopene and Lutein	• Immune cell integrity (via antioxidant defense)
Vitamin E	• Immune cell integrity (via antioxidant defense)
Vitamin C	• Lymphocyte proliferation • Neutrophil chemotaxis and phagocytosis • Immune cell integrity (via antioxidant defense)
Polyunsaturated fatty acids	• Immune cell chemotaxis and proliferation • Inflammatory cytokine production • Corticosteroid production

Although the mechanisms underlying exercise-associated immune changes are multifactorial, there are four key areas where exercise may directly contribute to altered immune function: 1) Reductions in plasma glutamine concentrations; 2) decreased plasma glucose concentrations; 3) increased production of free radicals and other reactive oxygen species; and 4) increased production of pro-inflammatory prostaglandins (Pedersen and Hoffman-Goetz 2000). Thus, nutritional supplementation of the athlete with glutamine, carbohydrate, antioxidants, or prostaglandin inhibitors may, in principle, provide a means of mitigating exercise-induced immunosuppression.

Glutamine Supplementation

Together with glucose, glutamine (a non-essential amino acid) is an important energy source for lymphocytes and monocytes (Table 3). Consequently, decreased levels of glutamine have resulted in reduced lymphocyte proliferation *in vitro* (Castell 2003). Skeletal muscle is the major tissue involved in glutamine production and is known to release glutamine into the bloodstream at a high rate (Newsholme 1994). Plasma glutamine concentration has been shown to decline in response to various stressors, including prolonged strenuous exercise (Rohde et al. 1998a, b; Kryzwkowski et al. 2001a, b). Furthermore, low glutamine levels have been described in athletes with “overtraining syndrome” (Castell 2003). It has been hypothesized that during intense physical exercise, the demand on muscle and other organs for glutamine is such that the immune system may be forced into a glutamine debt, which temporarily affects its function (Castell 2003). However, results on the ability of glutamine supplementation to ameliorate post-exercise immunosuppression have been equivocal. In a series of placebo-controlled field studies conducted in marathon runners, glutamine supplementation at 0.1 g/kg BW after competition resulted in faster restoration of circulating immune cells and a lower incidence of self-reported infections (Castell 2003). Glutamine supplementation at 0.25 to 0.9 g/kg BW following a marathon (Rohde et al. 1998a) or repeated bouts of bicycle ergometer exercise (Rohde et al. 1998b;

Kryzwkowski et al. 2001a,b) abolished the post-exercise decline in plasma glutamine, but did not influence the post-exercise impairment in specific measures of immune function.

Plasma glutamine has also been shown to be reduced in horses following exercise that simulated the road and tracks phase of a three-day event, as well as after sustained high intensity (115% VO_{2MAX}) exercise to fatigue (Routledge et al. 1999). Harris et al. (2006) demonstrated that plasma glutamine concentrations could be transiently increased in sedentary horses by feeding common dietary ingredients or by glutamine supplementation (either as glutamine or glutamyl-peptide). This finding is important, because glutamine is also the preferred fuel for intestinal enterocytes; thus, the ability to raise plasma glutamine levels in response to feeding or supplementation indicates at least some of the dietary supply will become available to immune cells outside the gut. However, it remains to be determined whether glutamine supplementation following exercise has any impact on exercise-associated declines in immune function in horses.

Carbohydrate Supplementation

Many aspects of exercise-induced immunosuppression seem to be caused by elevated levels of stress hormones (Pedersen and Hoffman-Goetz 2000). Therefore, nutritional strategies that effectively reduce the stress hormone response to exercise would be expected to limit the degree of exercise-induced immune dysfunction. It is well established that a reduction in blood glucose is linked to activation of the hypothalamic-pituitary-adrenal axis, resulting in increased release of cortisol and growth hormone, decreased release of insulin and variable effects on epinephrine. Given that prolonged exercise typically results in a decline in blood glucose, it has been suggested that consumption of carbohydrate during exercise could attenuate the rise in stress-related hormones and thereby diminish changes in immunity. This hypothesis has been tested in human athletes in a number of studies by Nieman and colleagues (see Nieman and Pedersen 1999). Ingestion of carbohydrate-containing beverages before, during (about 1 liter/hour) and after 2.5 hours of exercise was associated with higher plasma glucose levels, a reduced cortisol and growth hormone response, fewer perturbations in circulating immune cell counts, and prevention of the exercise-induced reduction in neutrophil and monocyte phagocytosis and oxidative burst activity and lymphocyte proliferation. More recently, carbohydrate beverage ingestion during a 3-hour treadmill run at 70% VO_{2MAX} was shown to reduce plasma levels of IL-6, as well as the anti-inflammatory cytokines IL-10 and IL-1ra, but did not affect gene expression of the more pro-inflammatory cytokines TNFα and IL-1β (Nieman et al. 2003). These data suggest that carbohydrate ingestion may attenuate the secondary, but not the primary pro-inflammatory cascade, which could decrease the need for immune responses related to anti-inflammation. Consumption of 30 to 60 grams of carbohydrate per hour during 2.5 hours of strenuous cycling prevented both the decrease in circulating Th1-cells and the suppression of INFγ from stimulated lymphocytes (Lancaster et al. 2005). As mentioned above, Th1-cells (and INFγ) are critical to antiviral defence and suppression of these cells may be an important mechanism leading to increased risk of infection after prolonged exercise (Gleeson 2007; Smith 2003). While carbohydrate feeding during exercise appears to be effective in minimizing some of the immune perturbations associated with prolonged continuous exercise, it seems less effective for less-demanding exercise of an intermittent nature or when prolonged exercise is performed to the point of fatigue (sees Gleeson 2006). Furthermore, it is not clear if the magnitude of effects observed with carbohydrate feeding during prolonged exercise is sufficient to reduce the risk of infection.

In the horse, there has been a considerable amount of study investigating carbohydrate supplementation with respect to reducing glycogen depletion during exercise and promoting glycogen repletion after exercise; however, the impact these practices have on immune function has not been addressed. In humans, the size of the glycogen stores in muscle and liver at the onset of exercise has also been shown to influence the hormonal and immune response to exercise. For example, Mitchell et al. (1998) observed that exercising for one hour in a glycogen-depleted state resulted in greater decreases in lymphocyte numbers following exercise in humans. Based on the promising findings in humans, the immunomodulating effects of carbohydrate supplementation, as well as the impact of reduced glycogen stores, deserves study in horses participating in endurance-type activities.

Antioxidant Supplementation

Immune cells are dynamic and highly metabolic, resulting in the production of reactive oxygen species as a part of normal cellular respiration. In addition, respiratory burst, a mechanism by which neutrophils and macrophages use to degrade and kill ingested pathogens also produces superoxide and hydrogen peroxide. Increased formation of reactive oxygen species also accompanies the dramatic rise in oxidative metabolism during exercise. Reactive oxygen species are capable of damaging a number of biomolecules, including cell membrane lipids, proteins and DNA, if not kept in check by antioxidants. Direct effects of reactive oxygen species on the immune system include inhibition of neutrophil chemotactic and bactericidal activity and NK cell cytotoxic activity and a reduction in T-cell and B-cell proliferation

(Gleeson 2006). In theory, antioxidant supplementation may neutralize the reactive oxygen species generated during exercise, thereby mitigating exercise-induced immunosuppression. In practice, however, there remain inconsistencies in the literature regarding supplementation and immune responses to exercise. Nieman et al. (1997) found no effect of vitamin C supplementation (1000 mg for 8 days) on immune response to 2.5 hours of running in humans. In contrast, daily supplementation of 600 mg vitamin C for 3 weeks prior to a 90-km ultra-marathon reduced the self-reported symptoms of upper respiratory tract infections in the 2 week period following the competition (Peters et al. 1993). A combination of fat- and water-soluble antioxidant vitamins does not appear to be more successful in attenuating the post-exercise infection risk that vitamin C alone. Daily supplementation with 500 mg vitamin C alone, 500 mg vitamin C plus 400 IU vitamin E, 300 mg vitamin C plus 300 IU vitamin E and 18 mg β-carotene, or placebo were provided to individuals for 3 weeks prior to a 90-km ultra-marathon (Peters et al. 1996) . A lower incidence of self-reported symptoms of upper respiratory tract infections was reported only for runners with the highest intake of vitamin C. Vitamin C supplementation has also been shown to augment the increase in lymphocyte counts after exercise and attenuate the increases in serum cortisol and pro-inflammatory cytokines in ultramarathon runners when ingested at 1000–1500 mg/day, but not at 500 mg/day (Peters et al. 2001). Supplementation of equine athletes with vitamin E and/or vitamin C has been investigated for potential mitigation of exercise-induced oxidative stress. However, the potential role of these and other antioxidants in attenuating perturbations in immunity following strenuous exercise have not been researched in horses.

Polyunsaturated Fatty Acid Supplementation

Two groups of polyunsaturated fatty acids (PUFA) are essential to the body: omega-6 (n-6) fatty acids, derived from linoleic acid; and omega-3 (n-3) fatty acids, derived from α-linolenic acid. These fatty acids cannot be synthesized by the body and therefore must be supplied in the diet. The biological activity of PUFA can, in part, be attributed to their conversion to eicosanoids, including prostaglandins, leukotrienes and thromboxanes. The n-6 fatty acid arachidonic acid yields the 2-series prostaglandins (e.g., PGE_2) and the 4-series leukotrienes (e.g., LTB_4), which modulate the intensity and duration of inflammatory immune responses (Calder et al. 2002). Although PGE_2 exhibits pro-inflammatory effects, including inducing fever, increasing vascular permeability and vasodilation, and enhancing pain and edema, PGE_2 has long been regarded as one of the most powerful immuno-suppressants (Smith 2003). In this respect, PGE_2 suppresses lymphocyte proliferation and NK cell activity and inhibits production of TNFα, IL-1, and IL-6 from macrophages and IL-2 and INFγ from Th1 cells (Calder et al. 2002). It has also been suggested that PGE_2 may play a role in exercise-induced immunosuppression by tipping the balance in favour of a Th2-type response (Smith 2003). Pedersen et al. (1990) reported that PGE_2 production by monocytes increased 270% following an acute bout of exercise. In addition, PGE_2 released from macrophage and neutrophils appears to be involved in post-exercise suppression of NK cells, the effects of which may persist for as long as 7 days after an event (Gannon et al. 1995).

The n-3 fatty acid, eicosapentaenoic acid (EPA), is a substrate for the synthesis of an alternate family of eicosanoids, the 3-series prostaglandins and the 5-series leukotrienes. In general, the EPA-derived eicosanoids are weaker inflammatory agents than those synthesized from arachidonic acid. In addition, EPA suppresses the production of eicosanoids from arachidonic acid by competing for placement in cell membranes and for the cycloxygenase and lipoxygenase enzymes responsible for oxidizing EPA and arachidonic acid to their respective eicosanoids. Consequently, dietary supplementation with n-3 fatty acids has been shown to modify immune and inflammatory responses in healthy sedentary individuals and chronic disease states (see Calder et al. 2002; Calder 2006; Sijben and Calder 2007). However, surprisingly little is known about the potential contribution of dietary fatty acids to the regulation of exercise-induced alterations in immunity. In fact, only one study appears to have addressed immunomodulation of fatty acids in relation to exercise. However, this study found that plasma levels of TNFα, IL-6, and IL-1ra and neutrophil and lymphocyte concentrations in male runners following a marathon were not altered in response to fish oil supplementation (providing 3.6 grams of n-3 fatty acids per day) for 6 weeks prior to competition (Toft et al. 2000).

Similar to the observations in other species, n-3 fatty acids in the form of flaxseed (rich in α-linolenic acid) or fish oil (rich in EPA) have been shown to modify biomarkers of inflammation and some aspects of immune function in sedentary horses (see review in NRC 2007). Research has also included the provision of n-3 fatty acids to exercising horses. However, the use of dietary fatty acids to mitigate immunosuppression following strenuous exercise has not yet been addressed in the horse.

Conclusions

The application of immunonutrition to the horse is a relatively new area of study. Yet interest in this area is gaining momentum as horse owners seek complementary and holistic methods to maintain the health of their horses. Further, many horses compete or participate in a variety of athletic events, which can challenge the immune system and increase

risk of infection. Therefore, nutrition and feeding strategies that help alleviate perturbations in immune function resulting from strenuous exercise and training are needed. In humans, dietary carbohydrate supplementation during and after prolonged continuous exercise has shown to mitigate many aspects of exercise-induced immunosuppression. Similarly, supplementation with antioxidants, particularly vitamin C alone or in combination with vitamin E, appears to bolster immune function and reduce symptoms of disease in athletes competing in strenuous events. Other immunomodulating nutrients (e.g., omega-3 fatty acids) have shown potential in sedentary subjects, but require greater evaluation in the exercising subject.

Ultimately, multiple endocrine and metabolic factors are involved in exercise-induced immune modulation. Therefore, it is unlikely that one single nutrient supplement will completely eliminate exercise-related immune dysfunction and risk of infection in performance horses. Synergistic effects between various immunomodulatory nutrients deserves further investigation in the athlete. Additionally, it is important to remember that mega-doses of nutrients can potentially impair immune function and have other toxic effects. Therefore, strategic provision of immunomodulatory nutrients should be undertaken with caution until research has confirmed safe and effective application in the horse.

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