Nutriceuticals and other drugs used to enhance fertility in stallions

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Abstract

Until recently, there has been little information regarding the ability to improve semen quality or fertility of stallions through alterations in diet. This paper presents evidence from work in humans and other species, including the stallion, which indicates that dietary supplementation with fatty acids, polyamines, vitamins or antioxidants may indeed have the potential of improving semen quality, particularly in those individuals who have marginal semen quality prior to supplementation.

Keywords: Stallion, semen quality, dietary supplementation, fatty acids

Introduction

Over the years, horsemen have been supplementing their animals' diets with various products in an attempt to enhance performance and overall well being. Most of these products have been geared toward improving stamina, hair coat, joint function and hoof growth. Historically, supplements touted to improve the breeding performance of stallions have not proven to be efficacious. Recently however, supplements have become available that show real promise in this regard.

Fatty Acids

Most fatty acids are straight-chain compounds, with an even number of carbon atoms. Chain-lengths can range from two to 80 but most commonly range from 12 up to 24. With a chain length from two to six, they are called short-chain, from eight to 10 they are called

medium-chain and 12 up to 24 they are called long-chain fatty acids. Among straight-chain fatty acids, the simplest are referred to as saturated fatty acids. They have no double bonds and cannot be altered by hydrogenation. When double bonds are present, fatty acids are said to be unsaturated. They are called monounsaturated fatty acids (MUFA) if only one double bond is present and polyunsaturated fatty acids **(**PUFA) when multiple double bonds are present.

The double bonds are counted from the methyl group determining the metabolic family, noted by n-x (with n being the total number of carbon and x the position of the last double bond). For example, linoleic acid is also named 18:2 n-6 in the shorthand nomenclature. Therefore this PUFA has 18 carbon atoms, 2 double bonds and there are 6 carbon atoms from the last double bond to the terminal methyl group. The number following "Omega-" in Omega-3 and Omega-6 fatty acids indicates the position of the first double bond, counting from the terminal methyl group on the molecule. Hence, linoleic acid (18:2 n-6) is an Omega-6 fatty acid. Omega-3 fatty acids cannot be converted to Omega-6 fatty acids or vice-versa.

Omega-3 fatty acids play an important role as structural membrane lipids in all cells. They are also precursors to reactive substances such as prostaglandins and leukotrienes, and possess anti-inflammatory, antiarrhythmic, antithrombotic and vasodilatory properties. There are also data to indicate that dietary supplementation with Omega-3 fatty acids to horses may modify the response to endotoxin by reducing the synthesis of potentially harmful cellular mediators. A plethora of equine dietary supplements containing Omega-3 fatty acids and their precursors are currently being marketed.

Semen from virtually all species examined contains relatively large amounts of lipid. Semen lipids play a major role in motion characteristics, sensitivity to cold shock and fertilizing

capacity of sperm. Phospholipids are the major lipid components found in semen and they are largely composed of PUFAs.[1] While spermatozoa from all mammals contain high concentrations of PUFAs,[1,2] the combination and distribution of PUFA in semen varies among species.[3] For example, the distribution of long chain PUFAs in stallion spermatozoa is more similar to boars than that of bulls or roosters.[3] Major differences in the lipid content of bull spermatozoa compared to those of boars and stallions are the relative amounts of 22:5 and 22:6 fatty acids. Spermatozoa of bulls have higher levels of 22:6 fatty acids whereas spermatozoa from stallions and boars have higher levels of 22:5 fatty acids.[3] Bulls and roosters produce spermatozoa that are very resistant to cold shock and freeze well, whereas spermatozoa from boars and stallions have very low tolerance to cold shock and in general, freeze poorly.

In particular, docosahexaenoic acid (DHA; 22:6 n-3, an Omega-3 fatty acid) is the major 22:6 PUFA in semen and docosapentaenoic acid (DPA; 22:5 n-6, an Omega-6 fatty acid) is the major 22:5 PUFA. The majority of seminal PUFAs reside in spermatozoa. In men with poor sperm motility, the level of DHA in seminal plasma as well as the ratio of Omega-3 to Omega-6 fatty acids in their spermatozoa was found to be significantly lower than in men with normal semen quality.[4] Studies in the boar and other species have shown that increasing the ratio of DHA to DPA in semen increases fertilizing capacity and semen quality.[2,5] Conversely, higher levels of DPA relative to DHA results in reduced fertility.[2] The potential role of DHA in spermatozoal motility and membrane stability is supported by the fact that membranes high in DHA are noted for their flexibility, compressibility, elasticity and deformability.[6]

Animals are unable to synthesize PUFAs from saturated or monounsaturated fatty acids. Therefore, they must acquire them from precursor PUFAs in their diet. Transport of PUFAs from the diet to semen has been shown to occur in a number of species including humans,[7] fowl,[5]

boars,[2] and rams.[8] Vegetable oils, such as corn and soybean oil, contain high levels of linoleic acid, the parent compound of DPA. Most proprietary equine rations are therefore, very high in linoleic acid, as well as other Omega-6 series fatty acids and their precursors while the precursors for Omega-3 fatty acids, such as DHA, are very low. A diet of this nature would favor the formation of DPA over DHA since conversion of precursors to DPA and DHA uses the same competitive enzymatic pathway. Since high DPA to DHA ratios in semen have been associated with reduced sperm quality and fertility, typical equine diets with an overwhelming availability of n-6 precursors could have a negative impact on quality of stallion semen and its tolerance to cooling and freezing.

While simply supplementing the stallion's diet with precursors to Omega-3 fatty acids such as cod liver oil or flaxseed oil can increase the overall level of Omega-3 fatty acids in semen, this may not result in the desired effects of improved semen quality. For example, supplementing boar diets with cod liver oil did not improve the freezability of semen.[9] However, when a supplement containing pre-formed DHA and antioxidants was added to boar rations, significant increases in semen quality and fertility were observed compared to boars fed a control diet.[2] Feeding the supplement to boars resulted in a number of benefits including a higher DHA to DPA ratio in semen, as well as an increase in total spermatozoal number, spermatozoal concentration, motility score, percentage of normal spermatozoa, and percentage of viable cells.

Cooling and freezing of spermatozoa can induce cellular injury, which is associated with a disruption of membrane lipids, resulting in damage to mitochondria and loss of integrity of both the plasma and acrosomal membranes. These events are accompanied by a loss of motility, viability and fertilizing capacity of sperm, a phenomenon commonly referred to as "cold shock". Differences in the ability of spermatozoa from various animals to resist cold shock appear to be

related to their sperm membrane lipid composition.[3] The lipid composition of spermatozoal membranes not only influences the response of spermatozoa to cooling and freezing, but also plays a major role in the physiologic changes leading to fertilization

Most breed registries have allowed the use of cooled, transported semen, and/or frozen-thawed semen for a several years. The use of shipped semen in the cooled-liquid or frozen state offers many advantages to breeders. Unfortunately, there are many stallions that produce semen that is unable to provide acceptable fertility after undergoing the rigors of cooling and storage, and cryopreservation magnifies this reduction in fertility even further. This problem and the promising results observed in pigs prompted equine researchers to investigate whether the addition of a DHA-enriched dietary supplement could result in improvements in equine semen quality.

Researchers at Texas A&M used eight stallions in a 2 x 2 crossover study to determine if feeding a supplement rich in DHA would improve semen quality.[10] The stallions were randomly assigned to one of two treatment groups (n = 4/group). Within these groups, the stallions were fed either their normal diet (control) or their normal diet top-dressed with 250 grams of a DHA-enriched supplement. The feeding trials lasted for 14 weeks after which a 14-week washout period was imposed, during which only the normal diet was fed. Following the wash out period, the treatment groups were reversed for another 14 week feeding trial. Feeding the supplement resulted in a 3-fold increase in semen DHA levels and a doubling of the ratio of DHA to DPA in the stallions' semen. Spermatozoal motion characteristics in fresh semen were unaffected by feeding the supplement and after 24 h of cooling and semen storage, total and progressive spermatozoal motility also did not differ between treatment groups. However, the spermatozoa from stallions fed the supplement exhibited higher velocity and straighter trajectory than those of

stallions being fed the control diet. Beneficial effects were more apparent after 48 hours of cooling and storage, where increases in the percentages of spermatozoa exhibiting total motility, progressive motility and rapid motility, were observed in the semen of stallions being fed the supplement. Total sperm numbers and percentage of spermatozoa with normal morphology were unaffected by treatment. However, when stallions were being fed the supplement, the sperm concentration in their ejaculates was almost double that of when they were fed the control diet. Therefore, it is possible that the observed improvements in semen quality after cooling and storage could be attributed, at least in part, to the reduced exposure of sperm to seminal plasma prior to and during processing.

In a subset of four stallions, whose progressive spermatozoal motility was <40% after 24 hours of cooling and storage when they were fed the control diet, feeding the supplement resulted in improvements in mean progressive spermatozoal motility after both 24 hours and 48 hours of cooled storage. Feeding the supplement resulted in similar improvements in motion characteristics being observed in frozen-thawed semen. Total spermatozoal motility, progressive spermatozoal motility, and percentage of spermatozoa exhibiting rapid motility were significantly higher in frozen-thawed semen of stallions being fed the supplement.

Despite increasing the level of DHA in semen by feeding the supplement, the level of DPA did not decline. The level of DPA in semen remained higher than that of DHA so that the DHA:DPA ratios were always less than one. Because the stallion's rations were typical equine formulations containing corn and soybean oils, which are the precursors that favor the pathway to DPA, the authors speculate that more dramatic improvements in semen quality may be observed if modifications in the main fat content of the diet are incorporated with the DHA supplement.

The authors concluded that supplementing the diet of highly fertile stallions or those that produce sperm that survive cooling does not appear warranted. However, stallions of marginal fertility and those whose spermatozoa have poor tolerance to cooling and freezing would be horses that might benefit most from being fed the supplement. Optimizing levels of DHA and its precursors by altering the diet of marginally fertile stallions, may improve their semen quality sufficiently enough to make them commercially viable for cooling or freezing.

Similar studies were carried out at the University of Arizona. In those studies, three stallions were fed 550 grams of a DHA-enriched supplement in addition to their normal diet for 90 days.[11] Dietary supplementation resulted in a 3.5-fold increase in semen DHA concentrations. Unlike the study performed at Texas A&M, motion characteristics of spermatozoa in cooled and frozen-thawed semen did not differ between supplemented and non-supplemented stallions. However, in this study dietary n-3 PUFA supplementation did result in a 46% increase in daily sperm output and a 15% increase in normal morphology. The most dramatic improvements were observed for one stallion which had the lowest percentages of morphologically normal and progressively motile spermatozoa prior to supplementation. Recently, workers in Uruguay[12] performed a 2 x 2 crossover study similar to the one performed at Texas A&M[10] with 3 stallions/group and treatment consisting of 30 grams of DHA being fed as a supplement for 80 days. In that study, dietary DHA supplementation improved total sperm numbers, sperm morphology and percentages of live sperm. Progressive motility in semen cooled for 48 hours and frozen-thawed semen improved for some stallions. Consistent with the other studies, the improvements were most noticeable for stallions that initially had poorer semen quality. Improvements in semen quality have also been reported for stallions whose diets were supplemented with rice oil.[13]

Polyamines

Both spermine and spermidine are polyamines found in all cells and thought to be essential for replication, growth, and differentiation.[14] It is believed that spermine and spermidine are produced by the prostate and found in the semen of most mammals. Early investigations documented increased spermatozoal motility *in vitro* when spermine was added to spermatozoa from mice, rats, guinea pigs and rabbits.[12] However, the exact physiologic role of spermine and spermidine in semen remains a matter of debate and their functions may be concentration dependent. Micromolar amounts of spermine in semen appear to enhance the acrosome reaction[15] while milimolar amounts appear to inhibit the acrosome reaction.[15,16]

In rams, ejaculates with spermatozoal motility greater than 85% had approximately two-fold higher spermine and total sperm polyamine content than ejaculates with lower motility. Compared to spermatozoa from lambs the spermatozoa of mature rams had approximately three-fold higher levels of spermidine, spermine, and total polyamines.[17] Lower levels of spermidine are found in the seminal plasma of men with idiopathic asthenozoospermia (poor spermatozoal motility) as well as those with asthenozoospermia associated with diabetes compared to normozoospermic men.[18]

To date, no studies have been published which examined the effects of dietary supplementation of polyamines on stallion semen. However, anecdotal information exists from practitioners using an herbal supplement called "SpermAid", which was originally marketed to increase fertility and libido in stallions, as well as to increase testicular hormone production. The active ingredients in this product are the phytochemicals spermine and spermidine, which are found in radish leaves, radish root, cucumber fruit, and oats. Feeding of the supplement is typically initiated three weeks prior to the breeding season. While significant improvements in

spermatozoal motility have not been reported with the use of this product, a number of slow breeding stallions have anecdotally shown dramatic improvements in libido.

Vitamins and antioxidants

Dietary supplementation with antioxidants and vitamins has been shown to have beneficial effect on semen quality. Many of these vitamins exert their beneficial effects through their antioxidant properties. However, as with most supplements examined, conflicting evidence exists regarding their efficacy. The conflicting results can be related to the species of the male subjects in the different studies as well as the doses of individual supplements or combination of supplements investigated.

Vitamins C and E are well known for their antioxidant properties and are those that have been the most extensively examined. In rabbits, dietary supplementation with vitamin C, vitamin E or a combination of vitamins C and E increased total spermatozoal output, spermatozoal concentration and spermatozoal motility while decreasing dead and abnormal spermatozoa in the ejaculate.[19] Analogous findings have been reported for humans and boars.[19,20] In humans, vitamin C was associated with higher spermatozoal numbers and concentrations in ejaculates, whereas vitamin E appeared to exert its effects by improving spermatozoal motility.[20] Similarly, there was tendency for semen production to be greater for boars supplemented with water soluble vitamins, with the effect being less in boars supplemented with fat soluble vitamins.[21] While the intake of high levels of antioxidant vitamins was associated with better semen quality, moderate intake did not appear to be effective.[20] While other investigators were unable to demonstrate any improvements in conventional semen quality parameters from infertile men,[22,23] supplementation with Vitamins C and E did result in a significant reduction in DNA fragmentation.[23]

Work in the stallion is more limited. In 1978, a German investigator gave 10 stallions of three different breeds an emulsion containing Vitamins A and E for eight weeks.[24] Improvements in ejaculate volume, spermatozoal concentration, spermatozoal morphology and spermatozoal motility, including post-thaw motility were reported. However, the improvements were not universal and appeared to differ among breeds. More recently, Russian workers formulated a complex feed additive that included vitamins A, D and E.[25] They reported improved spermatozoal motility in fresh semen and that spermatozoa remained viable longer after freezing and thawing.

Another antioxidant, showing promise for improving semen quality is L-carnitine (levocarnitine). Along with its antioxidant properties, L-carnitine is essential for mitochondrial energy metabolism. Both L-carnitine and L-acetyl-carnintine are found in high concentrations in the epididymis and both forms are accumulated by spermatozoa.[26] In men with asthenozoospemia, combined treatment with L-carnitine and L-acetyl-carnitine was effective in increasing spermatozoal motility.[24] The most significant improvements were seen in men with the lowest numbers of motile spermatozoa prior to treatment. Feeding L-carnitine to boars resulted in higher semen volumes and spermatozoal concentrations thereby increasing the total number of available spermatozoa in ejaculates for artificial insemination.[27]

Conclusions

It is clear that dietary alterations can have an effect on semen quality and in some cases, fertility. Controlled studies in stallions are few, but those investigating fatty acids, in particular Omega-3 fatty acids such as DHA, have shown real potential. Based on work in other species,

further studies involving optimal levels of individual supplements and combinations of supplements which could act synergistically to improve stallion semen quality are needed.

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