Calmatives for the Excitable Horse: A Review of L-tryptophan

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Abstract

Preparations that contain tryptophan are marketed world wide as calmative agents to treat excitable horses. Tryptophan is the amino acid precursor for serotonin, a neurotransmitter implicated in sedation, inhibition of aggression, fear and stress, in various animal species and humans. Experiments have shown that tryptophan supplementation decreases aggression in humans, dogs, pigs, poultry, and fish, and that it may reduce fearfulness and stress in calves, vixens and poultry. However, behavioural characteristics more closely linked to excitement, such as hyperactivity in dogs, are not modified by tryptophan supplementation. Research using a variety of animals other than horses, has shown that the behavioural response to tryptophan supplementation varies with age, breed and gender, and can be modified by diet, exercise, social status, and level of arousal. Significantly, the response is species-dependent, and there are no scientific publications that confirm the efficacy of tryptophan as a calmative in excitable horses. The few studies where tryptophan has been administered to horses suggest that low doses (relative to those contained in commercial preparations) cause mild excitement, whereas high doses reduce endurance capacity, and cause acute haemolytic anaemia if given orally, due to a toxic hindgut metabolite. As tryptophan continues to be used as an equine calmative, there is an urgent need for research to confirm its efficacy in horses, and to establish a safe therapeutic dose range. In the meantime, available data suggest that it would be imprudent to rely on tryptophan to calm the excitable horse, and instead, that a greater effort should be made to identify the underlying causes of excitability, and to explore more appropriate non-pharmacological remedies.

Introduction

Tryptophan supplements are marketed worldwide as calmatives for `excitable' horses. This paper outlines the proposed mechanism of action of tryptophan, and discusses nutritional factors that influence tryptophan uptake into the central nervous system. The few tryptophan experiments that have been conducted in horses are examined in detail, and tryptophan research in other species is reviewed, in search of evidence to support its use as a calmative agent.

Tryptophan use became highly controversial in 1989 when the US Food and Drug Administration (FDA) took action to limit its availability as a food supplement for humans. This was due to the association between the use of L-tryptophan supplements made by a Japanese Company, and a US epidemic of Eosinophilia Myalgia-Syndrome (EMS), involving more than 1500 reported cases, and at least 38 deaths (for review see Hertzman et al., 1991). It is hotly debated whether the epidemic was caused by L-tryptophan itself, or a contaminant of the commercial product, but while the FDA

restrictions on human use remain in place today, L-tryptophan continues to be used in the US and elsewhere in the world, as a supplement for animals.

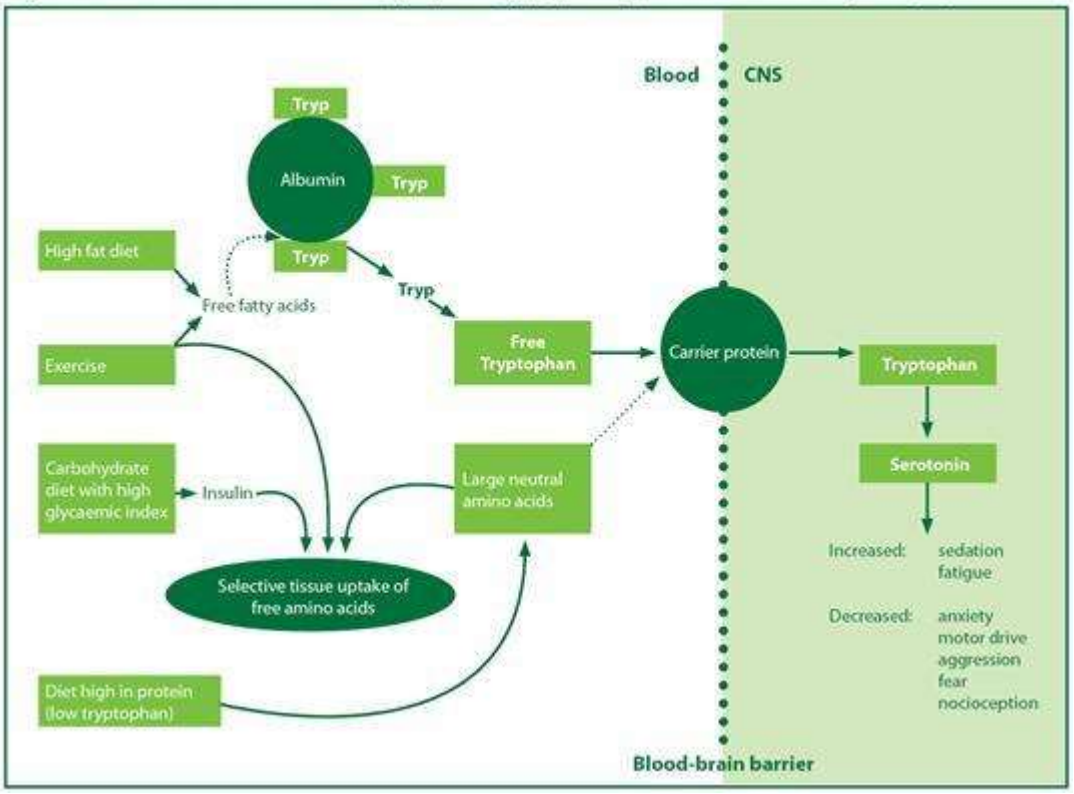
Several equine calmative preparations are available commercially that contain L-tryptophan, mostly in combination with other `calming' ingredients such as thiamine and magnesium. These preparations are sold as pastes or feed additive powders, with recommended tryptophan dosages ranging from 0.8 to 13 mg/kg. Depending on the manufacturer, claims of calmative efficacy are either express or implied, with at least one Australia-based manufacturer claiming that tryptophan can also enhance athletic performance (Nature Vet). Some manufacturers recommend everyday use (Hi Form), others propose daily supplementation for 2-3 days prior to a stressful event (Kelato Animal Health), and one company advocates usage 24 and 4 h prior to a stressful situation, such as a race (Nature Vet). One US manufacturer claims that "there are no known side-effects in horses from the supplementation of tryptophan" (Richdel Inc.).

Proposed Mechanism and Rationale for Use of Tryptophan

Tryptophan is an essential amino acid and the precursor of serotonin. An increase in this neurotransmitter in the brain has been associated in numerous studies with an rise in sleepiness or sedation, and decreased aggression, fearfulness, mania, insomnia and pain sensitivity (Liebermann et al., 1983; Leathwood, 1987; Mench and Shea-Moore, 1995; Nakanishi et al., 1998; Winberg et al., 2001), with some exceptions (Bagshaw et al., 1994; Mench and Shea-Moore, 1995; Janczak et al., 2001; Rouvinen et al., 1999). Tryptophan is converted to serotonin via a two-step reaction. The first and rate-limiting step is the conversion of L-tryptophan to 5-hydroxytryptophan, catalysed by tryptophan hydroxylase (Boadle-Biber, 1993). This enzyme is only found in serotonin-forming cells, whereas the second reaction in the two-step conversion, the decarboxylation of 5-hydroxytryptophan, can occur in any mammalian cell (Lovenberg et al., 1962, 1968). Under normal conditions in mammals, tryptophan hydroxylase is not fully saturated by its substrate, and no negative feedback mechanism from serotonin is known. Theoretically, therefore, increased concentrations of tryptophan in the CNS equate to increased serotonin synthesis (Fernstrom and Wurtman, 1972).

Hence, the rationale for tryptophan use in horses is that increasing the plasma concentration of tryptophan will lead to increased tryptophan uptake by the CNS, and increased serotonin production in the brain. However, even without tryptophan administration, several nutritional factors can influence the passage of tryptophan across the blood-brain barrier, and alter serotonin production (Fig. 1). It is important to understand these factors to be able to evaluate critically the results of tryptophan experiments.

Figure 1. Effects of diet and exercise on tryptophan (Tryp) uptake by the central nervous system (CNS).



Tryptophan enters the CNS by active transport across the blood-brain barrier, sharing a carrier protein with other large neutral amino acids (LNAA) such as leucine, isoleucine, methionine, valine, phenylalanineand tyrosine (Fernstrom and Wurtman, 1972). Because of competition between tryptophan and the LNAA for carrier binding sites, the rate at which tryptophan enters the brain is a function of both tryptophan and LNAA concentrations. In humans, for example, a fourfold increase in the ratio of plasma tryptophan to LNAA, results in double the concentration of tryptophan in the brain, and a 20% increase in brain serotonin (Leath-wood, 1987). The relative plasma concentration of LNAA can be increased by feeding a high protein diet that contains more LNAA than tryptophan, or may be decreased via the release of insulin, which appears to remove selectively the LNAA from plasma with less effect on tryptophan (Clark and Mills, 1997). Thus, Lyons and Truswell (1988) showed a positive association in humans, between diets with a high glycaemic index, insulin concentrations, and the ratio of tryptophan to LNAA in plasma. Fernstrom and Wurtman (1971) observed an increase in brain serotonin after feeding a carbohydrate meal to rats.

Unlike most amino acids, tryptophan circulates in the plasma bound extensively to plasma proteins, chiefly albumin (McMenamy and Oncley, 1958). This binding is believed to restrict the tissue uptake of tryptophan in response to insulin (Lipsett et al., 1973), but also limits the competition between tryptophan and the LNAA for transport into the CNS. Estimates of the level of tryptophan binding vary from 20% to 90%, depending in part on the concentration of free fatty acids, which compete with tryptophan for binding to albumin. An increase in free fatty acids following a high fat meal for example, can result in elevated free plasma tryptophan, and ultimately, more serotonin production (Newsholme et al., 1987). This phenomenon is part of the `central fatigue hypothesis', a theory based on the observation that exercise promotes an increase in plasma free fatty acids, and a decrease in plasma LNAA due to uptake by skeletal muscle. As both conditions favour more tryptophan entering the CNS, increased production of brain serotonin might be expected, and this could account for the decreased

motor drive and increased sensation of fatigue, experienced after a period of strenuous exercise (Newsholme et al., 1987).

The role of tryptophan as a cause of central fatigue is not universally accepted. Indeed, there is a history of controversy over the central and peripheral factors that cause muscle fatigue (for review see Gandevia, 2001). For many physiologists, it was convenient to assume that the only important changes are those that occur in muscle. Substrate supply is particularly important, and glycogen loading can enhance endurance (Green et al., 1995). However, through advanced techniques such as Trans-Cranial Magnetic Stimulation, it has been shown convincingly, that between 10% and 20% of muscle fatigue in humans originates in the CNS (Taylor and Gandevia, 2001). Furthermore, both peripheral and central fatigue seem to have multiple causes. The neurotransmitters associated with central fatigue may include noradrenaline, dopamine and GABA, while humoral factors that might signal changes to the CNS, could include cortisol, glutamine, and cytokines such as interleukin-6 (Gandevia, 2001).

The role of serotonin (and hence tryptophan) in central fatigue, is supported partly by evidence for a "thermal switch" in the hypothalamus, which limits exercise performance once the core temperature reaches a critical threshold (Gandevia, 2001). In humans, serotonergic neurones project into the hypothalamus (Tork and Hornung, 1990), and exercise endurance deteriorates, when the environmental temperature is high (Pitsiladis and Maughan, 1999). A link between serotonin and thermal regulation was shown in men and women, who were made to exercise during heat stress. Endurance was prolonged when the participants were fed dietary supplements containing branched chain amino acids (Mittleman et al., 1998). Presumably, the supplements limited tryptophan entry in to the CNS, and reduced serotonin synthesis.

Other evidence for the role of tryptophan/serotonin in central fatigue, includes the observation that exercise increases tryptophan concentrations and serotonin turnover in various parts of the CNS (Chaouloff et al., 1986). Furthermore, exercise endurance can be improved with serotonin antagonists, and declines with serotonin ago-fists (Davies, 1995), or inhibitors of serotonin re-uptake (Wilson and Maughan, 1992; Bailey et al., 1993, Davies, 1995). For reviews on the association between serotoninand exercise, see Jacobs and Fornal (1993), or Chaouloff (1997).

In summary, the type and level of dietary fat, protein, and carbohydrates, as well as the exercise status of a horse, could all influence tryptophan uptake into the CNS, and the response to tryptophan administration. Experiments designed to evaluate tryptophan as a calmative have not always considered these factors.

Effects of Tryptophan in Horses

Despite its popular use as a `natural' calmative for horses, few scientific studies of tryptophan have been conducted in this species, and only one has focused on behavioural effects. Bagshaw et a]. (1994) administered L-tryptophan to 10 mature Arabian and Standardbred mares subjected to isolation stress. The amino acid was given orally at two doses (0.05 and 0.1 mg/kg), and the horses were isolated in an enclosed stall. Two to four hours after tryptophan dosing, isolated horses given 0.1 mg/kg had higher heart rates and exhibited more walking-sniffing activity than controls. Mares given the lower dose also showed more activity when in visual contact with other mares.

Bagshaw et al. (1994) concluded that at the level administered, tryptophan had no sedative effect, but seemed to cause a mild excitation (Bagshaw et al., 1994). This was likened to the `serotonin behavioural syndrome' described in rats by Mokler et al. (1992). This condition is characterised by front-paw treading, hindlimb abduction, head weaving, and tail-lashing, and is reported to occur when

low doses of tryptophan are given, possibly associated with a failure to saturate tryptophan hydroxylase.

The study by Bagshaw et al. (1994) was criticised by Clark and Mills (1997) on several grounds. Clark and Mills implied that the background diet used in Bagshaw's study was not tightly controlled. They also argued that the use of mature mares was inappropriate because the blood-brain barrier becomes less permeable to tryptophan with age (although no evidence was given to support this assertion in horses). Thirdly, results for the behavioural variable (activity) were said to be ambiguous, because increased locomotion can reflect changes in diet selection and appetite, as well as stress. Clark and Mills (1997) suggest that changes in serotonin could induce a preference for lower carbohydrate feeds, and increase food-seeking behaviour.

The short duration of Bagshaw's study should also be considered in view of findings in other species, that behavioural changes are not observed less than a week after commencing daily tryptophan supplementation (Laycock and Ball, 1990; Winberg et al., 2001). However, perhaps the most clinically significant aspect of Bagshaw's study is that the level of L-tryptophan administered was much lower than that contained in most commercial preparations, and no change was seen in blood concentrations of tryptophan or serotonin in the treated horses. Nevertheless, as Bagshaw et al. (1994) point out, studies in other species have also failed to detect increased blood levels, despite increased brain concentrations of tryptophan and serotonin after oral dosing (Fernstrom and Wurtman, 1972, 1974).

Finally, Bagshaw et al. (1994) reported that the two Arabian mares used in the study were more active and vocal in isolation, and had lower blood serotonin concentrations than the eight Standardbred mares. In a separate study, Arabian mares were found to have lower blood serotonin concentrations than Swedish Warm-blood mares housed on the same property, and fed the same diets (S.L. Ralston, unpublished data). This implies an association between serotonin and behaviour in horses, and suggests that there may be breed differences in the behavioural response to tryptophan administration.

In contrast to Bagshaw's low-dose tryptophan study, Paradis et al. (1991a) explored the toxic effects of tryptophan at high concentrations. A group of adult Shetland Ponies given a single dose of tryptophan (600 mg/kg) by stomach tube, exhibited signs of restlessness, elevated respiratory rate, haemolysis, and haemoglobinuria. A second group treated once orally with a lower dose of tryptophan (350 mg/kg) showed less severe haemolysis, but also presented with increased respiratory rates within 24 h of dosing. Serum concentrations of bilirubin were increased, and findings consistent with haemoglobinuric nephrosis were evident at necropsy.

However, a third group of ponies given a single intravenous (IV) infusion of tryptophan (100 mg/kg) showed no clinical abnormalities, haemolysis, or haemoglobinuria (Paradis et al., 1991a). Although the IV dose was the lowest used, plasma tryptophan concentrations were elevated to the same extent as seen following oral treatment. Paradis et al. (1991a) concluded that oral administration of tryptophan leads to the formation of a haemolytic metabolite in the digestive tract, possibly indole. A similar effect is seen in cattle, where rumen microorganisms convert tryptophan to 3-methylindole, which causes severe acute respiratory disease (fog fever'). Respiratory symptoms are not observed in cattle after IV infusion of tryptophan (Carlson et al., 1968), or after oral dosing with rumen-protected tryptophan (Nakanishi et al., 1998). It is not known how much oral tryptophan reaches the hind gut in horses and undergoes microbial metabolism, but it is known that the conversion of tryptophan to indole (but not 3-methyl indole) occurs in caecal fluid in vitro (Paradis et al., 1991a), and that severe haemolysis occurs following oral dosing with indole (Paradis et al., 1991b).

In the study by Paradis et al. (1991a), no signs of sedation were reported in ponies given the tryptophan infusion, while those treated orally became restless. While this brings into question the efficacy of tryptophan as a calmative in horses, it should be noted that the doses used were between 27 and 750 times higher than those found in commercial preparations.

Furthermore, the study was conducted on a small number of animals (four horses per group), which is less than optimal for behavioural work.

The third and most recent paper on this topic focused on the central fatigue hypothesis, and the effects of tryptophan on exercise capacity in horses (Farris et al., 1998). Endurance times were recorded in seven mares exercised to fatigue on a high-speed treadmill, and treated with tryptophan and (or) glucose infusions in a cross-over design. When the mares were not given tryptophan, they showed a reduction in muscle glycogen concentrations with exercise, and responded to glucose infusions with better endurance. This shows the importance of peripheral factors in causing fatigue, particularly substrate supply, as discussed earlier (Green et al., 1995). Furthermore, glucose infusions caused no change in plasma free fatty acid concentrations, or in the ratio of free tryptophan to LNAA, which is not consistent with the central fatigue hypothesis proposed by Newsholme et al. (1987).

Nevertheless, when the mares were given tryptophan (100 mg/kg), their endurance was reduced significantly (' 16%), which is consistent with the effect seen when rats were treated with serotonin agonists (Davies, 1995), and supports a role for tryptophan and serotonin in contributing to central fatigue. Furthermore, glucose infusions caused no improvement in endurance when tryptophan was given. Thus, while substrate supply may be the most important limiting factor in the normal exercising horse, Farris et al. (1998) showed that by causing central fatigue, tryptophan can reduce endurance, regardless of substrate supply.

Apart from inducing central fatigue, serotonin has been shown to have peripheral effects consistent with impaired exercise performance. Bailey et al. (1993) noted that after exhaustive treadmill exercise, rats previously given a serotonin agonist had significantly higher muscle glycogen concentrations than those given a placebo, or a serotonin antagonist. Similarly, in the study by Farris et al. (1998), there was a tendency for the mares to use less muscle glycogen during exercise, if they were given tryptophan. Hence, it is possible that tryptophan and (or) serotonin, may prevent the utilisation of muscle glycogen during prolonged exercise, thereby reducing endurance capacity due to an inability to utilise this energy substrate.

Thus, at high doses tryptophan may reduce endurance capacity, which would have adverse consequences for horses involved in stamina-related pursuits such as racing, endurance and eventing. High doses of tryptophan also induce haemolysis if given orally, compromising the health of the animal. Low doses of tryptophan may be excitatory in the horse, and no scientific studies have shown that tryptophan is an effective calmative in horses when used in the dose range provided by commercial preparations. For evidence that tryptophan supplements may be beneficial in this regard, we are compelled to extrapolate from research conducted in other species.

Effects of Tryptophan in Non-Equine Species

The actions of tryptophan have been studied extensively in humans and other species. Experiments in humans have focused on depression and aggression (Liebermann et al., 1986; Leathwood, 1987; Young,1991; Bellisle et al., 1998), sleep (Hartmann, 1986), and dietary interactions (Liebermann et al., 1986; Bel-lisle et al., 1998). Animal studies have focused on reducing stress, fearfulness and aggression, especially in production situations (Laycock and Ball, 1990; Bagshaw et al., 1994; Mench

and Shea-Moore, 1995; Nakanishi et al., 1998; Warner et al., 1998; Rouvinen et al., 1999; DeNapoli et al., 2000; Janczak et al., 2001; Winberg et al., 2001).

Tryptophan supplementation is reported to reduce depression in mice (Hilakivi-Clarke et al., 1990), to increase the feeling of well-being in humans suffering depression (Liebermann et al., 1986), and to reduce the incidence of self-mutilation in caged monkeys (Mench and Shea-Moore, 1995). Although these observations indicate an effect of tryptophan on mood or behaviour, they are of limited relevance to the treatment of excitable horses, and will not be explored further here (for reviews, see Liebermann et al., 1986; HilakiviClarke et al., 1990; Mench and Shea-Moore, 1995).

More relevant to horses, is evidence that tryptophan may affect fearfulness. This judgement is based partly on changes in exploratory behaviour. Unfortunately, such changes seem to be sex and species-dependant, and are interpreted differently by different investigators. For example, newly-weaned calves spend more time lying down when supplemented with tryptophan, with less exploratory behaviour and vocalisation (Nakanishi et al., 1998). The investigators interpreted this as reduced stress and fearfulness. Newberry and Blair (1993), who observed that tryptophan-supplemented chickens flap less when handled, reached a similar conclusion.

However, Janczak et al. (2001) interpreted reduced exploratory behaviour in tryptophan-treated mice, as increased fearfulness. Rouvinen et al. (1999) report that tryptophan also tends to reduce exploratory behaviour in male silver foxes, whereas the opposite effect is seen in female foxes. In agreement with Janczak et al. (2001), but in contrast to Nakanishi et al. (1998), Rouvinen et al. (1999) associate increased exploratory behaviour in females with enhanced confidence and decreased fearfulness.

Extreme fearfulness can lead to hysteria, which can be a significant problem in flocks of layer hens, causing a marked effect on egg production. Laycock and Ball (1990) fed a diet containing 5 g tryptophan/kg feed to a commercial layer flock of 10,000 hens suffering from hysteria, and showed that the symptoms were abolished two days after the cessation of a six-day course of treatment. Feed intake increased, and a 23% increase in egg production was observed over the supplementation period. Hysteria gradually returned after the tryptophan supplementation ceased, although it was eliminated without return when in a subsequent experiment, tryptophan was administered to the entire barn, rather than just one room.

Increased concentrations of tryptophan, serotonin and associated metabolites, were also found in the brains of tryptophan-supplemented birds (Laycock and Ball, 1990).

Tryptophan depletion and the consequent lowering of brain serotonin have been linked to increased aggression in humans (Young, 1991), whereas tryptophan supplements have been used with some success in treating pathologically aggressive people (Young, 1991). Furthermore, although tryptophan was reported to potentiate territorial aggression in male mice (Lasley and Thurmond, 1985), the majority of studies report decreased aggression in a variety of animal species. Thus, in male chickens, tryptophan supplementation decreases aggressive pecking, especially in the more dominant birds (Shea et al., 1991). Reduced territorial behaviour and aggressive dominance is also seen in dogs given tryptophan (DeNapoli et al., 2000). Pigs subjected to pre-slaughter stress perform fewer aggressive acts if supplemented with 5 g tryptophan/kg feed (~55 mg/kg body weight) for five days pre-slaughter, but with no reduction in the incidence of PSE meat (Warner et al., 1998). Similarly, rainbow trout supplemented with dietary L-tryptophan (0.15% and 1.5% of feed wet weight) perform fewer aggressive acts than controls, when faced with a small intruder (Winberg et al., 2001).

In the last study, aggression was reduced in trout after seven days of daily supplementation, but not after three days (Winberg et al., 2001). This delayed response is consistent with results obtained by Laycock and Ball (1990) in layer hens, where it took six days for tryptophan to alleviate hysteria. Because tryptophan would be expected to increase serotonin synthesis and release quite rapidly (i.e., within a few hours), Winberg et al. (2001) postulated that the effects of tryptophan on aggression may involve an additional mechanism, involving the slower conversion of serotonin to melatonin. In earlier studies, Munro (1986) demonstrated that aggression was inhibited in cichlid fish injected with serotonin, but that this effect was inhibited if S-adenosyl homocysteine (a substance that inhibits the conversion of serotonin to melatonin) was also injected.

Finally, the effects of tryptophan on hyperactivity may be especially relevant to its use as a calmative in horses. For example, the behavioural criteria for assessment of hyperactivity in dogs include: excessive pacing or circling, not remaining in sit-stay or down-stay positions when required, being easily distracted by extraneous stimuli, impulsive behaviour (not waiting), limited attention span, excessive vocalisation, and not heeding commands (DeNapoli et al., 2000). Such behaviours resemble those that an excitable horse may display in a stressful situation. In the study by DeNapoli et al. (2000) using dogs, tryptophan reduced aggression, but did not reduce hyperactivity. This is consistent with the results of earlier studies in rats (Callaway et al., 1993) and children (Halpern et al., 1994; Popper, 1997), where hyperactivity was not reduced by treatment with selective inhibitors of serotonin re-uptake.

Factors that Influence the Response to Tryptophan

In behavioural science, it is acknowledged that various animal species can respond differently to the same compound, and that even within species, responses can be influenced by factors such as breed, gender, social status, and level of arousal (Mench and Shea-Moore, 1995). Accordingly, great care must be taken when extrapolating to horses, the results of tryptophan studies conducted in humans, or other animal species.

There is a lack of direct evidence to support the use of tryptophan in horses, but the owner or veterinarian may be persuaded that tryptophan does have calmative properties, from experience, or the weight of anecdotal evidence. If tryptophan is to be used, it is well to consider the many factors that could influence the efficacy of a supplement.

The amount and type of fat, protein and carbohydrate in a horse's diet should all be considered. A low fat diet producing a low concentration of free fatty acids in the blood, would increase the capacity of albumin to mop up any free tryptophan as it enters the circulation, reducing tryptophan efficacy. A diet that contains more fat might have a calmative effect itself, which could either enhance or mask any effect of tryptophan supplementation. Compared with horses fed mostly roughage, a greater calmative effect may be seen when tryptophan is given to horses on concentrate diets, for two reasons. First, some horses become more excitable when fed concentrate diets, so the effects of a calmative would be more noticeable. Secondly, carbohydrate diets with a high glycaemic index cause more insulin release, more uptake of LNAA into peripheral tissues, and less competition with tryptophan for uptake into the brain (Fig. 1). Thus, Leathwood (1987) argues that combined with a carbohydrate load, tryptophan is an effective sedative at doses that are ineffective when combined with a protein meal. Finally, the concentration of tryptophan in the protein component of a horse's diet, relative to the concentration of other large neutral amino acids, will determine whether supplementing with additional tryptophan is likely to provide any benefit.

Clark and Mills (1997) have cautioned that age may decrease the response to tryptophan, because of decreased permeability of the blood-brain barrier. Bagshaw et al. (1994) noted the possibility that

there are breed differences in the basal level of serotonin activity in horses, whereas sex differences in hypothalamic serotonin concentrations have been confirmed in pigs, such that female swine have less serotonin and are more sensitive to changes in the tryptophan:LNAA ratio than males (Henry et al., 1992, 1996). This could account for the sex difference in the response to tryptophan noted in the fox by Rouvinen et al. (1999), and indicates that gender could be a critical factor in the response of horses.

The concentration of brain serotonin is also known to vary within breed and gender, according to social status. Generally, subordinate individuals have higher levels of brain serotonin and serotonin metabolites than dominant individuals (Mench and Shea-Moore, 1995), although the reverse may be true in primates. (Raleigh et al., 1984) reported that dominant monkeys had higher blood concentrations of serotonin than subordinates. Serotonin concentrations fell when the dominant monkeys were isolated, but rose again rapidly when they were re-introduced to the group and re-established their dominant position. Mench and Shea-Moore (1995) explained this apparent anomaly by suggesting that in dominant individuals, more tryptophan may be metabolised peripherally, and as such less is available for conversion to serotonin in the brain.

Finally, the level of arousal could influence the response of horses to tryptophan, as it has been shown in cats that arousal is associated with a higher rate of firing of serotonergic neurones (Trulson and Jacobs, 1979). Accordingly, Young (1991) has predicted that the effects of tryptophan would be greatest when animals are aroused, as would be expected in the case of the excitable or stressed horse. Nevertheless, considering species differences and the range of factors that may influence the response to tryptophan, it would be imprudent to guarantee a tryptophan calmative response in any individual.

Clinical Issues

It is difficult to recommend a therapeutic, or even a safe dose rate for using L-tryptophan in horses as a calmative. Based on a review by Leathwood (1987) of human clinical studies, tryptophan is an effective sedative at 500 mg (-7 mg/kg) if given with a carbohydrate load. Otherwise, a dose of 1 g or higher is required to consistently reduce sleep latency in people with mild insomnia. The usual clinical dose in humans is about 2 g (-28 mg/kg; Leathwood, 1987), which on a body weight basis, is more than twice that provided by any commercial equine preparation.

Whereas finisher pigs appeared to tolerate 5 g tryptophan per day for five days (Warner et al., 1998), several participants in a human study reported headache and severe nausea after ingesting a single 5 g dose (Greenwood et al., 1975). In other human studies, 10 g of tryptophan has been ingested (-140 mg/kg) without reported adverse effects (Bellisle et al., 1998).

As discussed earlier, extrapolating the results of monogastric studies to horses could be dangerous because of toxic hindgut metabolites. Thus, toxic effects on the blood and respiratory system occur in ponies following oral tryptophan at 350 mg/kg (Paradis et al., 1991a). An IV dose of 100 mg/kg has no adverse effects on haematology in ponies, but neither does it cause any obvious calmative effect (Paradis et al., 1991a), and reduces exercise endurance in mares (Farris et al., 1998). Furthermore, this may not be a safe oral dose, due to conversion in the hindgut to indole (Paradis et al., 1991a). At the other end of the spectrum, a dose of 0.1 mg/kg appears to be too low, causing mild excitation in horses (Bagshaw et al., 1994).

We are not aware of any studies on the long-term use of supplemental dietary tryptophan in horses, however it seems not to be harmful at 2 g/day in humans (Leath-wood, 1987), or at 5 g/kg feed in chickens (Laycock and Ball, 1990).

Final Considerations

We advocate caution in predicting the effects of tryptophan based on observations in other species, and have listed numerous factors that could influence the response of any individual. The absence of direct scientific evidence that tryptophan does calm excitable horses, is also of concern. However, two other issues should be considered when contemplating the suitability of tryptophan for this application.

First, excitability needs to be described or diagnosed accurately. Whereas McCann et al. (1988) characterised nervous or highly-reactive horses as those with a high activity index (horses that spend more time walking, trotting, cantering and galloping, than `normal' horses), Fraser (1992) described hyper-reactivity (or hyperactivity) in terms of aggression (mobile aggression, mobile alarm, threatening, biting, kicking) and aversion. Perhaps a closer consensus on what constitutes excitability (fearfulness versus mobility or aggression) is needed, before the effects of tryptophan can be properly assessed.

The second issue concerns identifying the cause of excitable behaviour. Among other factors, excitability has been linked to feeding rapidly fermentable carbohydrates (Greiwe et al., 1989; Kohnke, 1998), or surplus dietary energy (Fraser, 1992). In contrast, Holland et al. (1996) found that activity and reactivity were reduced in horses consuming a diet containing 10% corn oil. Thus, better dietary management could be more appropriate than tryptophan supplementation in many cases. In other circumstances, the needs of excitable horses may be served better by a variation in the exercise regime or housing conditions (Fraser, 1992).

Alternative training methods and more thorough acclimatisation of horses to potentially stressful situations and environments (e.g., competitions) may reduce the incidence and severity of excitability in horses. Perhaps it is basic measures such as these, which provide the most reliable, efficacious, and ethically acceptable solutions to problems associated with `excitability' in horses. In the words of Janczak et al. (2001) "The dilemma of whether our ethical obligations to animals demand that we provide environments which suit their ethological needs, rather than pharmaceutically providing them with the illusion of good welfare (Mench and Shea-Moore, 1995), will remain beyond the time when the effects of such substances are fully understood".

Conclusions

While tryptophan supplementation appears to be effective in reducing aggression and possibly fearfulness in some species, its impact on hyper-reactivity and stress are questionable. Few studies on tryptophan have been conducted in horses, and none has produced direct evidence of calmative efficacy in this species. It is known, however, that the effects of tryptophan on behaviour are species dependant, as well as being subject to the influence of diet, exercise, age, gender, breed, social status, and level of arousal.

Oral tryptophan at high doses causes haemolysis in ponies, due to the production in the gut of a toxic metabolite (Paradis et al., 1991a). Moreover, tryptophan infusion has been shown to reduce endurance in horses (Farris et al., 1998), and this appears to be at variance with the claims of at least one supplement manufacturer. The lack of information which establishes a safe and effective oral dose for L-tryptophan in horses, is of considerable concern.

Based on the available evidence, it would be incautious to rely on tryptophan supplementation to calm the excitable horse, especially when other measures might be available to address the underlying cause of excitability, such as altering diets, management and husbandry procedures.

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References

Bagshaw, C.S., Ralston, S.L., Fisher, H., 1994. Behavioural and physiological effect of orally administered tryptophan on horses subjected to acute isolation stress. Applied Animal Behaviour Science 40, 1-12.

Bailey, S.P., Davis, J.M., Ahlborn, E.N., 1993. Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue. Journal of Applied Physiology 74, 3006-3012.

Bellisle, F., Blundell, J.E., Fantino, M., Fern, E., Fletcher, R.J., Lambert, J., Roberfroid, M., Specter, S., Westenhofer, J., WesterpPlantenga, M.S., 1998. Functional food science and behaviour and psychological functions. British Journal of Nutrition 80, S173-S193.

Boadle-Biber, M.C., 1993. Regulation of serotonin synthesis. Progress in Biophysics and Molecular Biology 60, 1-15.

Callaway, C.W., Wing, L.L., Nichols, D.E., 1993. Suppression of behavioural activity by norfenfluramine and related drugs in rats is not mediated by serotonin release. Psychopharmacology 111, 169178.

Carlson, J.R., Dyer, I.A., Johnson, R.J., 1968. Tryptophan-induced interstitial pulmonary emphysema in cattle. American Journal of Veterinary Research 29, 1983-1989.

Chaouloff, F., 1997. Effects of acute physical exercise on central serotonergic systems. Medicine and Science in Sports and Exercise 29, 58-62.

Chaouloff, F., Laude, D., Guezennec, Y., Elghozi, J.L., 1986. Motor activity increases tryptophan, 5-hydroxyindoleacetic acid and homovanillic acid in ventricular cerebrospinal fluid of the conscious rat. Journal of Neurochemistry 46, 1313-1316.

Clark, J., Mills, D.S., 1997. Design considerations for the evaluation of tryptophan supplementation in the modification of equine behaviour. In: Mills, D.S., Heath, S.E., Harrington, L.J. (Eds.), Proceedings of the First International Conference on Veterinary Behavioural Medicine. Universities Federation for Animal Welfare, Potters Bar, pp. 164-173.

Davies, J.M., 1995. Carbohydrates, branched-chain amino acids, and endurance: the central fatigue hypothesis. International Journal of Sports Nutrition 5, S29-S38.

DeNapoli, J.S., Dodman, N.H., Shuster, L., Rand, W.M., Gross, K.L., 2000. Effect of dietary protein content and tryptophan supplementation on dominance aggression, territorial aggression, and hyperactivity in dogs. Journal of the American Veterinary Medical Association 217, 504-508.

Farris, J.W., Hinchcliffe, K.W., McKeever, K.H., Lamb, DR., Thompson, D.L., 1998. Effect of tryptophan and of glucose on exercise capacity of horses. Journal of Applied Physiology 85, 807816.

Fernstrom, J.D., Wurtman, R.J., 1971. Brain serotonin content: increase following ingestion of carbohydrate diet. Science 174, 1023-1025.

Fernstrom, J.D., Wurtman, R.J., 1972. Brain serotonin content: regulation by plasma neutral amino acids. Science 178, 414-416.

Fernstrom, J.D., Wurtman, R.J., 1974. Control of brain serotonin levels by the diet. Advances in Biochemistry and Psychopharmacology 11, 133-141.

Fraser, A.F., 1992. Abnormal and anomalous behaviour. The Behaviour of the Horse. Cab International, Oxford, pp. 205-213. Gandevia, S.C., 2001. Spinal and supraspinal factors in human muscle fatigue. Physiological Reviews 81, 1725-1789.

Green, H.J., Ball-Burnett, M., Symon, S., Grant, S., Jamieson, G., 1995. Short-term training, muscle glycogen, and cycle endurance. Canadian Journal of Applied Physiology 20, 315-324.

Greenwood, M.H., Lader, M.H., Kantameneni, B.D., Curzon, G., 1975. The acute effects of oral (-)-tryptophan in human subjects. British Journal of Clinical Pharmacology 2, 165-172.

Greiwe, K.M., Meacham, T.N., Fontenot, J.P., 1989. Effect of added dietary fat on exercising horses. Proceedings of the Equine Nutrition and Physiology Society 11, 101.

Halpern, J.M., Sharma, V., Siever, L.J., 1994. Seretogenic functions in aggressive and nonaggressive boys with attention deficit hyperactivity disorder. American Journal of Psychiatry 151, 243-248.

Hartmann, E.L., 1986. Effect of L-tryptophan and other amino acids on sleep. Nutrition Reviews (Suppl.) 44, 70-73.

Henry, Y., SEve, B., Colleaux, Y., Ganier, P., Saligaut, C., Jego, P., 1992. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs, in relation to plasma free amino acids and hypothalamic serotonin. Journal of Animal Science 70, 1873-1887.

Henry, Y., SEve, S., Mounier, A., Ganier, P., 1996. Growth performance and brain neurotransmitters in pigs as affected by tryptophan, protein, and sex. Journal of Animal Science 74, 27002710.

Hertzman, P.A., Falk, H., Kilbourne, E.M., Page, S., Shulman, L.E.,

1991. The eosinophilia-myalgia-syndrome - The Los-Alamos Conference. Journal of Rheumatology 18, 867-873.

Hilakivi-Clarke, L.A., Durcan, M.J., Lister, R.G., Linnoila, M., 1990. Effect of tryptophan on the behaviour of nonstressed and stressed mice in Porsolt's swim test. Pharmacology Biochemistry and Behaviour 37, 273-276.

Holland, J.L., Kronfeld, D.S., Meacham, TN., 1996. Behaviour of horses is affected by soy lecithin and corn oil in the diet. Journal of Animal Science 74, 1252-1255.

Jacobs, B.L., Fornal, C.A., 1993. 5-HT and motor control: a hypothesis. Trends in Neuroscience 16, 346-352.

Janczak, A.M., Bakken, M., Braastad, B.O., 2001. Cautionary note regarding the use of nutritional L-tryptophan to alter aversion-related behaviour in mice. Applied Animal Behaviour Science 72, 365-373.

Kohnke, J.B. (Ed.), 1998. Feeding and Nutrition of Horses: The Making of a Champion. Birubi Pacific, Rouse Hill, New South Wales, p. 23.

Lasley, S.M., Thurmond, J.B., 1985. Interaction of dietary tryptophan on territorial aggression, motor activity, and neurochemistry in mice. Psychopharmacology 87, 313-321.

Laycock, S.R., Ball, R.O., 1990. Alleviation of hysteria in laying hens with dietary tryptophan. Canadian Journal of Veterinary Research 54, 291-295.

Leathwood, P.D., 1987. Tryptophan availability and serotonin synthesis. Proceedings of the Nutrition Society 46, 143-156.

Liebermann, H.R., Corkin, S., Spring, B.J., Gordon, J.H., Wurtman, R.J., 1983. Mood, performance and pain-sensitivity: changes induced by food constituents. Journal of Psychiatric Research 17, 135-145.

Liebermann, H.R., Spring, B.J., Garfield, G.S., 1986. The behavioural effects of food constituents: strategies used in studies of amino acids, protein, carbohydrate and caffeine. Nutrition Reviews (Suppl.) 44, 61-70.

Lipsett, D., Madras, B.K., Wurtman, R.J., Munro, H.M., 1973. Serum tryptophan level after carbohydrate ingestion: selective decline in non albumin-bound tryptophan coincident with reduction in serum free fatty acids. Life Sciences 12, 57-64.

Lovenberg, W., Jequier, E., Sjoerdsma, A., 1968. Tryptophan hydroxylation in mammalian systems. In: Garattini, S., Shore, P.A. (Eds.), Advances in Pharmacology A, 6. Academic Press, New York, pp. 21-36.

Lovenberg, W., Weissbach, H., Udenfriend, S., 1962. Aromatic L-amino acid decarboxylase. Journal of Biological Chemistry 237, 89-92.

Lyons, P.M., Truswell, A.S., 1988. Serotonin precursor influenced by type of carbohydrate meal in healthy adults. American Journal of Clinical Nutrition 47, 433-439.

McCann, J.S., Heird, J.C., Bell, R.W., Lutherer, L.O., 1988. Normal and more highly reactive horses I. Heart rate, respiration rate and behavioural observations. Applied Animal Behaviour Science 19, 201-214.

McMenamy, R.H., Oncley, J.L., 1958. The specific binding of Ltryptophan to serum albumin. Journal of Biological Chemistry 233, 1436-1447.

Mench, J.A., Shea-Moore, M.M., 1995. Moods, minds and molecules: the neurochemistry of social behaviour. Applied Animal Behaviour Science 44, 99-118.

Mittleman, K.D., Ricci, M.R., Bailey, S.P., 1998. Branched-chain amino acids prolong exercise during heat stress in men and women. Medicine and Science in Sports and Exercise 30, 83-91.

Mokler, D.J., Sullivan, S.A., Winterson, B.J., 1992. Behaviours induced by 5-hydroxytryptophan in neonatal, preweaning, post-weaning and adult Sprague-Dawley rats. Pharmacology, Biochemistry and Behaviour 42, 413-419.

Munro, A.D., 1986. Effects of melatonin, serotonin and naloxone on aggression in isolated cichlid fish (Aequidens pulcher). Journal of Pineal Research 3, 257-262.

Nakanishi, Y., Shigemori, K., Yanagita, K., Mieno, M., 1998. Behavioural and growth effect of oral administration of rumen protected tryptophan on weanling beef calves. Memoirs of the Faculty of Agriculture Kagoshima University 34, 89-95.

Newberry, R.C., Blair, R., 1993. Behavioural responses of broiler chickens to handling: effects of dietary tryptophan and 2 lighting regimens. Poultry Science 72, 1237-1244.

Newsholme, E.A., Acworth, I.N., Blomstrand, E., 1987. Amino acids, brain neurotransmitters and a functional link between muscle and brain that is important in sustained exercise. In: Benzi, G. (Ed.), Advances in Myochemistry. Libby Eurotext, London, pp. 127-138.

Paradis, MR., Breeze, R.G., Bayly, W.M., Counts, D.F., Laegreid, W.W., 1991a. Acute hemolytic anemia after oral administration of L-tryptophan in ponies. American Journal of Veterinary Research 52, 742-747.

Paradis, M.R., Breeze, R.G., Laegreid, W.W., Bayly, W.M., Counts, D.F., 1991b. Acute hemolytic anemia induced by oral administration of indole in ponies. American Journal of Veterinary Research 52, 748-753.

Pitsiladis, Y.P., Maughan, R.J., 1999. The effects of exercise and diet manipulation on the capacity to perform prolonged exercise in the heat and in the cold in trained humans. Journal of Physiology (London) 517, 919-930.

Popper, C.W., 1997. Antidepressants in the treatment of attention deficit/hyperactivity disorder. Journal of Clinical Psychiatry 58, S14-S29.

Raleigh, M.J., Mcguire, M.T., Brammer, G.L., Yuwiler, A., 1984. Social and environmental influences on blood serotonin concentrations in monkeys. Archives of General Psychiatry 41, 405-410.

Rouvinen, K., Archbold, S., Laffin, S., Harri, M., 1999. Long-term effects of tryptophan on behavioural response and growing-furring performance in silver fox (Vulpes vulpes). Applied Animal Behaviour Science 63, 65-77.

Shea, M.M., Douglass, L.W., Mench, J.A., 1991. The interaction of dominance status and supplemental tryptophan on aggression in Gallus domesticus males. Pharmacology, Biochemistry and Behaviour 38, 587-591.

Taylor, J.L., Gandevia, S.C., 2001. Transcranial magnetic stimulation and human muscle fatigue. Muscle and Nerve 24, 18-29.

Tork, I., Hornung, J.P., 1990. Raphae nuclei and the serotonergic system. In: Paxinos, G. (Ed.), The Human Nervous System. Academic Press, San Diego, pp. 1011-1022.

Trulson, M.E., Jacobs, B.L., 1979. Raphe unit activity in freely moving cats: correlation with level of behavioural arousal. Brain Research 169, 135-150.

Warner, R.D., Eldridge, G.A., Hofineyr, C.D., Barnett, J.L., 1998. The effect of dietary tryptophan on pig behaviour and meat quality - preliminary results. Australia Society of Animal Production 22, 325.

Wilson, W.M., Maughan, R.J., 1992. Evidence for a possible role of 5hydroxytryptamine in the genesis of fatigue in man: administration of paroxetine, a 5-HT re-uptake inhibitor, reduces the capacity to perform prolonged exercise. Experimental Physiology 77, 921924.

Winberg, S., OVerli, 0., Lepage, 0., 2001. Suppression of aggression in rainbow trout (Oncorhynchus mykiss) by dietary L-tryptophan. Journal of Experimental Biology 204, 3867-3876.

Young, S.N., 1991. Some effects of dietary components (amino acids, carbohydrate, folic acid) on brain serotonin synthesis, mood and behaviour. Canadian Journal of Physiology and Pharmacology 69, 893-903.