Papers

Effect of diet on plasma tryptophan and serotonin in trained mares and geldings

D. Alberghina, C. Giannetto, E. K. Visser, A. D. Ellis

Concentrations of tryptophan (TRP) and serotonin (5-HT) in plasma were measured in 36 moderately trained Dutch warmblood horses after eight weeks on a high fibre (n=18) or high starch (n=18) diet. Samples were taken three hours after feeding, when the horse was at rest, either at 11.00 or 14.00 hours. Plasma 5-HT and pH were significantly higher in horses fed a high fibre diet than those fed a high starch diet ($P<0.05$ and $P<0.01$, respectively), and significantly higher levels of TRP were found in mares than geldings ($P<0.05$). Plasma 5-HT may therefore be a good marker of serotonergic activity.

THE effects of a high concentrate versus high fibre diet on performance parameters and welfare of the horse have been widely studied in the past (Tinker and others 1997, Nadeau and others 2000, McGreevy and others 2001, Ellis and others 2002). Horses fed diets low in 'structural' fibre have a greater susceptibility to develop stereotypical behaviour (apparently functionless, repetitive activities, such as wind sucking or crib biting) and stomach ulcers due to a lack of time spent chewing, reduced saliva production and a negative effect on the hindgut environment (Nicol and others 2002, Ellis and others 2003). Gillham and others (1994) reported that stereotypical behaviour significantly increased during and after feeding concentrates. The mechanism by which diet may influence behaviour could be clarified by the hypothesis that diet influences the serotonergic system (Grimmett and Sillence 2005). In human beings, this hypothesis has been confirmed in women but not in men (Walsh and others 1995). The neurotransmitter serotonin (5-HT) is known to be involved in many physiological and behavioural processes, including mood, appetite, sleep, sexual behaviour and cognition, but also in the pathophysiology of psychiatric disorders such as depression, anxiety and obsessive compulsive disorder (OCD) (Lucki 1998). Crib biting and wind sucking in horses have been described as stereotypical behaviours similar to OCD in people (Shuster and Dodman 1998). 5-HT is synthesised via tryptophan (TRP) hydroxylase and therefore TRP levels may be an indicator of 5-HT metabolism. Diets with different levels of protein and carbohydrates could influence plasma TRP levels. Modification of this essential amino acid may influence the rate of synthesis of the corresponding neurotransmitter (Fernstrom 1983). The depletion or increase of TRP in diets has been shown to affect behaviour and mood in experimental conditions in rats and human beings (Blokland and others 2002, Markus and others 2005, Jans and others 2007). Limited evidence indicates that TRP supplementation at sufficient levels could have a calming effect on horses, as shown in other species (Mench and Shea-Moore 1995, Winberg and others 2001); however, low levels of TRP supplementation have an excitatory effect (Grimmett and Sillence 2005) and no calming effect of TRP supplementation was seen on resting horses subjected to acute isolation stress (Bagshaw and others 1994).

In a study by Lebelt and others (1998), crib biting horses had lower plasma 5-HT levels than control group horses, and a preliminary study by Alberghina and others (2006) showed a tendency of lower plasma 5-HT levels in horses fed with a low fibre diet than horses fed with a high fibre diet. There is a common perception that high concentrate diets make a horse more 'fizzy' (excitable/nervous), but there is limited scientific proof for this. The authors hypothesised that the effect of diet on the serotonergic system could be evaluated by plasma TRP and 5-HT activity. On the basis that recent studies suggest that major neurotransmitters, including 5-HT, affect lactate production, especially during stress (Uehara and others 2008), blood parameters such as lactate and pH levels were also investigated. The aim of this study was to compare the effect of two isoenergetic diets with different forage:concentrate ratios on plasma TRP, 5-HT, lactate and pH levels, and to evaluate the influence of time of feeding and sex on the parameters studied.

Veterinary Record (2010) 166, 133-136 doi: 10.1136/vr.c502

D. Alberghina, DVM, PhD,
C. Giannetto, DVM, PhD,
Department of Experimental Sciences and Applied Biotechnology, Faculty of Veterinary Medicine, University of Messina, 98168 Messina, Italy
E. K. Visser, BSc, MSc, PhD,
Animal Sciences Group, Wageningen University and Research Centre, PO Box 65, 8200 AB Lelystad, The Netherlands
A. D. Ellis, BSc, PhD,
School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Southwell, Nottinghamshire NG25 0QF

E-mail for correspondence: dalberghina@unime.it

Provenance: not commissioned; externally peer reviewed

Materials and methods

The present study forms part of a larger study on behaviour and exercise in horses, which was carried out in three consecutive phases, according to three exercise and diet levels (light, medium and medium-hard) (Ellis and others 2003). Each phase lasted four weeks. In week 4 of each phase, a set exercise test (SET) was performed. The research reported here took place at the end of the second phase (after eight weeks on treatment). For phase 2 only (medium work), resting blood samples taken before the SET were analysed to determine of plasma TRP and 5-HT levels.

Subjects and management

Thirty-six Dutch warmblood horses (18 mares and 18 geldings) of three years of age and a mean (sd) bodyweight of 554 (42) kg were used in the study. Horses were clinically healthy, of ideal body condition score and based at the Research Institute for Animal Husbandry, Lelystad, the Netherlands. All procedures involving animal handling and testing were approved by the Animal Care and Use Committee of the Animal Sciences Group of Wageningen University and Research Centre in Lelystad.

Preparation

Horses had become accustomed to individual housing and work on the training mill and treadmill during an adaptation period of four months, reaching a light fitness level. They were then randomly assigned to two treatment groups, with equal numbers of each sex in each group, based on two isoenergetic diets containing either high levels of structural fibre with a concentrate:haylage ratio of 1:3 (n=18) or high levels of starch, with a concentrate:haylage ratio of 3:1 (n=18). The purpose of the two diets was to mimic two feeding regimens currently used for leisure and sports horses. The term 'high fibre' in the context of this study refers to a high component of structural fibre in the diet, which has not been mechanically chopped or ground.

Diet and exercise

Horses were kept on a light exercise regimen for phase 1 (the first four weeks) and moved up to a medium workload at the beginning of phase 2. The diet consisted of haylage (210 g crude fibre, 50 g starch and sugar, and 560 g dry matter per kg feed), or a high starch concentrate pellet formulated for performance horses (120 g crude fibre, 250 g starch and sugar, and 880 g dry matter per kg feed). Horses on the high fibre diet were also fed a mineral supplement in order to reach a level of mineral intake similar to horses given the high starch feed. Haylage was given twice daily, at 8.00 and 17.00, while concentrate feed was divided into three equal meals per day. All horses were fed above protein requirements (high fibre 1.4 times and high starch 1.9 times the digestible crude protein requirements) (Central Veeroden Beureau [CVB] 1996).

In phase 2, the mean fresh matter intake for horses on a high starch diet was 6.1 kg concentrates and 3.5 kg haylage. The mean fresh matter intake for horses on a high fibre diet was 2.3 kg concentrates and 11.9 kg haylage. Energy requirements were calculated according to a medium work level based on the Dutch Net Energy Evaluation System, equating to 1.4 times the maintenance requirements (CVB 1996). Horses had free access to fresh water and were exercised daily on a mechanical training mill (horse walker, 40 m circular) for 45 minutes (22 minutes walking, 15 minutes trotting and eight minutes cantering). On the last three days of week 4, resting blood samples were taken before a SET at 11.00 and 14.00 hours. Half of the horses from each treatment group performed the exercise test in the morning and half in the afternoon.

Blood samples

Resting samples were taken from the jugular vein three hours after feeding at 11.00 or 14.00 hours before SET, with an equal distribution between diet and sex per sampling period.

Venous blood was collected into a K_3-EDTA tube for 5-HT and TRP, and into a heparin tube for blood lactate, pH and haematocrit analysis. The K_3-EDTA tubes were centrifuged at 4°C (2500 *g* for 10 minutes) to obtain platelet-low plasma that was kept at –20°C until the analysis by high-performance liquid chromatography. Blood lactate, pH and haematocrit were analysed using an electrolyte/metabolite analyser (Environmental Measurements Laboratory).

Statistical analysis

All results were expressed as mean (se). Following testing for normal distribution (Kolmogorov-Smirnov test), a *t* test for quality of means was used for the comparison of parameters in each diet group. A one-way analysis of variance was applied to compare the influence of diet, time of feeding (morning or afternoon) and sex. The relationship between parameters was evaluated using Pearson's correlation coefficients. The significance level was set at P<0.05. Data were analysed using the software STATISTICA 5.5 (Statsoft).

Results

There were no differences in haematocrit between diet groups or subgroups according to the time of feeding or sex (haematocrit 33 per cent [se 0.6]). Levels of plasma 5-HT and blood pH were significantly higher in horses on the high fibre diet than those on the high starch diet (P<0.05 and P<0.01, respectively) (Table 1). Levels of TRP were very similar and lactate was slightly higher in horses on the high starch diet but the difference was not significant. There was no significant difference in 5-HT between morning and afternoon, but TRP, lactate and pH levels were significantly higher in the afternoon test results (P<0.01, P<0.001 and P<0.05, respectively) (Table 2).

Table 3 shows the interactions between diet or sex. Mares in the high fibre group had significantly higher TRP levels than geldings in the high fibre group (P<0.05). Overall, TRP was significantly higher in mares than in geldings (P<0.05), but only a weak trend of higher 5-HT levels was observed (Fig 1).

TABLE 1: Mean (se) serotonin (5-HT), tryptophan (TRP), lactate and pH levels in horses fed a high fibre or high starch diet

Parameter	High fibre (n=18)	High starch (n=18)	P value
5-HT (nmol/l)	168 (9.5)	133 (9.6)	<0.05
TRP (µmol/l)	65.08 (4.01)	65.91 (3.87)	NS
Lactate (mmol/l)	0.73 (0.06)	0.80 (0.05)	NS
pH	7.41 (0)	7.39 (0)	<0.01

NS Not significant

TABLE 2: Mean (se) serotonin (5-HT), tryptophan (TRP), lactate and pH levels in horses fed a high fibre or high starch diet, tested at different times of the day

Parameter	High fibre (n=18) Morning (n=9)	High fibre (n=18) Afternoon (n=9)	High starch (n=18) Morning (n=9)	High starch (n=18) Afternoon (n=9)	P
5-HT (nmol/l)	156 (11)	179 (17)	127 (31)	139 (16)	NS
TRP (µmol/l)	61 (4.3)	76 (4.6)*	54 (4.3)	71 (6.2)	<0.05
Lactate (mmol/l)	0.55 (0.03)	0.99 (0.07)†	0.68 (0.07)	0.84 (0.05)	<0.001
pH	7.41 (0)	7.41 (0)	7.40 (0)	7.38 (0)*‡	<0.01

* P<0.05 v morning
† P<0.005 v morning
‡ P<0.01 v afternoon

Discussion

In the present study, the effect of two different diets was examined in adult geldings and mares. The TRP levels within each diet were not assessed, but both diets were representative of ingredients and feed levels (volume) used for performance and leisure horses. Horses on the high fibre diet had significantly higher 5-HT levels than high starch-fed horses (P<0.02), although they were fed lower total crude protein; this supports findings from Alberghina and others (2006). Different plasma 5-HT concentrations could have been due to a different control in uptake of 5-HT in platelets by the 5-HT transporter (SERT), either due to a decreased synthesis or to an increased catabolism of 5-HT. It is not clear whether pH could have been involved in these mechanisms. The activity of human platelet SERT is not affected by transmembrane pH differences (Rudnick and others 1989).

TRP, which is a 5-HT precursor, is hydroxylated to 5-hydroxy-L-TRP (5-HTP) via the enzyme TRP hydroxylase. This enzyme occurs in two isoforms responsible for the non-neuronal (Walther and others 2003) and neuronal (Zhang and others 2004) synthesis of 5-HT,

TABLE 3: Mean (se) serotonin (5-HT), tryptophan (TRP), lactate and pH levels in horses according to sex and whether they were fed a high fibre or high starch diet

Parameter	Mares (18) High fibre (9)	Mares (18) High starch (9)	Geldings (18) High fibre (9)	Geldings (18) High starch (9)	P
5-HT (nmol/l)	178 (12)	141 (11)	158 (15)	120 (16)*	<0.05
TRP (µmol/l)	75 (4.9)	69 (6.4)	54.65 (4.26)*	63.06 (4.36)	<0.05
Lactate (mmol/l)	0.81 (0.07)	0.74 (0.09)	0.78 (0.06)	0.72 (0.1)	NS
pH	7.41 (0)	7.41 (0)	7.41 (0)	7.39 (0)†‡	<0.01

* P<0.05 v HF mares
† P<0.01 v HF mares
‡ P<0.05 v HF geldings

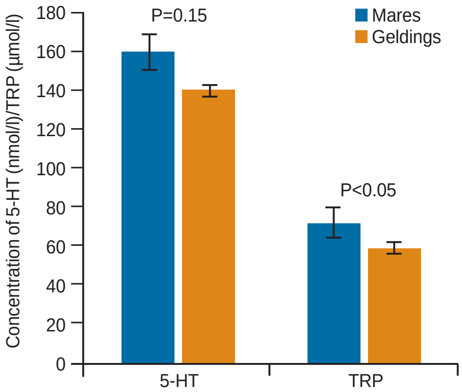

FIG 1: Mean (se) serotonin (5-HT) and tryptophan (TRP) levels in horses according to sex

and this reaction constitutes the rate-limiting step in 5-HT synthesis. The blood-brain barrier is impermeable to peripheral 5-HT, but not to 5-HTP, which does not require a specific transport protein (Rahman and others 1982). Diet could have affected the concentrations of cofactors for hydroxylation of TRP, such as vitamin B_3, vitamin B_6 and magnesium (Boadle-Biber 1993). Further research is necessary to clarify whether diet affects plasma 5-HT through pH modification or other factors. The dietary differences suggest that plasma 5-HT could be used to estimate changes in central 5-HT activity in the horse, as seen from human data (Sarrias and others 1990) and this needs to be investigated further. There was no influence of diet on resting plasma TRP and blood lactate levels. TRP in plasma is mainly bound to albumin. This binding is believed to restrict the tissue uptake of TRP in response to insulin (Lipsett and others 1973) but also limits the competition between TRP and large neutral amino acids for transport in the CNS. In foals, diet significantly influences blood glucose and gastrin concentrations, but insulin levels and the TRP:large neutral amino acid ratio do not differ significantly (Wilson and others 2007). The authors did not analyse free plasma TRP; however, Fernstrom and Fernstrom (2006) demonstrated a dissociation of free TRP and brain TRP pools. In a study of diurnal change, with blood collection every four hours for 48 hours, Piccione and others (2005), reported an increase of serum TRP in mares between 12.00 and 16.00 hours. TRP results from the present study showed a similar pattern between 10.00 and 14.00.

Diet and sex did not affect lactate levels. In a previous study comparing diets with cation-anion differences, Mueller and others (2001) did not find changes in blood lactate. The mean concentration of lactate measured in the present study was within the same range. Concentrations of basal blood lactate were significantly higher in afternoon samples ($P<0.001$). When measuring 24-hour rhythmicity of haematological parameters in athletic and sedentary mares, no effect of time of day on plasma lactate levels was detected by Piccione and others (2004). In the present study, one reason for the increase in lactate levels in the group from which samples were taken in the afternoon may be the nutritional status at this time. Horses sampled in the afternoon had received a lunch of only concentrates, while horses sampled in the morning had received a breakfast of haylage and concentrates. This would have affected horses on the high starch diet differently from horses on the high fibre diet that still had some haylage left over from the morning. Interestingly, it is horses on the high fibre diet that showed a greater increase in lactate levels in the afternoon, which may be related to increased hindgut fermentation due to high feed volume. Energy metabolism and availability of blood glucose may also have affected blood lactate values. The similar increase of TRP and lactate in the afternoon might suggest a relationship between these parameters. In adult horses, oral TRP supplementation significantly decreases blood lactate concentration after exercise (Vervuert and others 2005), while infusion of TRP in exercising horses leads to a reduction in endurance time (Farris and others 1998). Metabolism of TRP is complex and 5-HT is neither the major, nor the only, TRP metabolite; kynurenine, indole acetic acid and indole lactic acid are also important metabolites of TRP and an increase in their concentrations has been reported after TRP infusion in men (Heuther and others 1992).

The pH was lower in the high starch group ($P<0.01$), mainly due to a drop in pH in the afternoon, and this may reflect diet and nutritional status, although when considering lactate levels the opposite effect might be anticipated, as lactate was higher in the afternoon samples. When the pH was lower, lower 5-HT levels were recorded, showing a weak correlation ($R^2=0.39$), but there was no correlation with lactate.

A difference in the availability of neurotransmitter precursors due to the influence of sex hormones has been found in male and female human beings under the influence of sex steroids (Giltay and others 2008). Although no stallions were used in this study, there is still a difference in hormonal systems between mares and geldings in this respect. Solomon and Herman (2009) document that oestrogenic and 5-HT systems may interact differently to regulate mood in females and males. In the equine industry, the majority of male horses are geldings and it was therefore of interest to establish whether differences between mares and geldings were observed. Mares had higher 5-HT levels and significantly higher TRP levels ($P<0.05$) than geldings. Interestingly, mares had lower TRP on the high starch versus high fibre diet, whereas geldings had higher TRP on the high starch versus high fibre diet, indicating a difference in effect of diet according to sex. Behavioural observations showed that mares had a much greater tendency to eat bedding and faeces, which was significantly more prevalent in the high starch group (Ellis and others 2006). Many studies of animals and humans have confirmed sexual dimorphism for 5-HT (Maes and Meltzer 1995). Higher levels of TRP and 5-HT in females have been found in several brain structures (Carlsson and Carlsson 1988). Plasma 5-HT is also higher in women than in men (Ortiz and others 1988) and 5-HT activity is enhanced in females, possibly reflecting greater synthesis and turnover (Casper 1998). Oestrogen has been reported to increase the production of TRP hydroxylase in non-human primates (Bethea and others 2000), and Walsh and others (1995) reported that men are less likely to exhibit diet-enhanced alterations in brain 5-HT function than women.

This study was limited by the inability to produce a crossover design; however, a good-sized homogeneous sample population was used and results reflect the applied situation in the field when high fibre diets are replaced with high starch diets as workload increases. The results agree with previous work suggesting that plasma 5-HT may be a good marker of serotonergic activity. The implications of this on the behaviour of horses need to be established further.

Acknowledgements

The authors thank the caretakers of the horses at the experimental farm 'Waiboerhoeve' in Lelystad and thank the many students involved for their hard work. The study was supported by a grant from the Dutch Ministry of Agriculture, Nature and Food Quality and this sub-project was supported by research funds from Messina University.

References

ALBERGHINA, D., BRUSCHETTA, G., CAMPO, G. M., CAMPO, S., MEDICA, P. & FERLAZZO, A. M. (2006) Influence of diet and sex differences on plasma serotonin and tryptophan of trained thoroughbreds. In Management of Lameness Causes in Sport Horses: Muscle, Tendon, Joint and Bone Disorders. Ed A. Lindner. Wageningen Academic Publishers. pp 149-152
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
BETHEA, C. L., GUNDLAH, C. & MIRKES, S. J. (2000) Ovarian steroid action in the serotonin neural system of macaques. *Novartis Foundation Symposium* **230**, 112-130
BLOKLAND, A., LIEBEN, C. & DEUTZ, N. (2002) Anxiogenic and depressive like symptoms, but no cognitive deficits after acute tryptophan depletion in the rat. *Journal of Psychopharmacology* **16**, 39-49
BOADLE-BIBER, M. C. (1993) Regulation of serotonin synthesis. *Progress in Biophysics and Molecular Biology* **60**, 1-15
CARLSSON, M. & CARLSSON, A. (1988) A regional study of sex differences in rat brain serotonin. *Progress in Neuro-psychopharmacology and Biological Psychiatry* **12**, 53-61
CASPER, R. C. (1998) Serotonin, a major player in the regulation of feeding and affect. *Biological Psychiatry* **44**, 795-797
CVB (1996) Het definitieve VEP- en VREp-systeem. Documentatierapport 15. CVB
ELLIS, J. M., HOLLANDS, T. & ALLEN, D. E. (2002) Effect of forage intake on bodyweight and performance. *Equine Veterinary Journal Supplement* **34**, 66-70
ELLIS, A. D., MERKENS, H. W., VISSER, E. K., SCHILDER, M. B. H. & VAN REENEN, C. G. (2003) Effects of high concentrate versus high fibre diets on equine

digestive parameters in relation to performance, animal behaviour and welfare. Proceedings of the 54th Annual Meeting of the European Association of Animal Science. Rome, August 31 to September 3, 2003. p 416
ELLIS, A. D., VISSER, E. K. & VAN REENEN, C. G. (2006) The effect of a high fibre versus low fibre diet on behaviour and welfare in horses. Proceedings of the 40th International Congress of the International Society for Animal Ethology. Bristol, August 8 to 12, 2006. p 82
FARRIS, J. W., HINCHCLIFF, K. W., MCKEEVER, K. H., LAMB, D. R. & THOMPSON, D. L. (1998) Effect of tryptophan and of glucose on exercise capacity of horses. *Journal of Applied Physiology* **85,** 807-816
FERNSTROM, J. D. (1983) Role of precursor availability in control of monoamine biosynthesis in brain. *Physiological Reviews* **63,** 484-546
FERNSTROM, J. D. & FERNSTROM, M. H. (2006) Exercise, serum free tryptophan, and central fatigue. *Journal of Nutrition* **136,** 553S-559S
GILLHAM, S. B., DODMAN, N. H., SHUSTER, L., KREAM, J. A. & RAND, W. (1994) The effect of diet on cribbing behaviour and plasma β-endorphin in horses. *Applied Animal Behaviour Science* **41,** 147-153
GILTAY, E. J., BUNCK, M. C., GOOREN, L. J., ZITMAN, F. G., DIAMANT, M. & TEERLINK, T. (2008) Effects of sex steroids on the neurotransmitter-specific aromatic amino acids phenylalanine, tyrosine, and tryptophan in transsexual subjects. *Neuroendocrinology* **88,** 103-110
GRIMMETT, A. & SILLENCE, M. N. (2005) Calmatives for the excitable horse: a review of L-tryptophan. *Veterinary Journal* **170,** 24-32
JANS, L. A. W., LIEBEN, C. K. J. & BLOKLAND, A. (2007) Influence of sex and estrous cycle on the effects of acute tryptophan depletion induced by a gelatin-based mixture in adult wister rats. *Neuroscience* **147,** 304-317
HUETHER, G., HAJAK, G., REIMER, A., POEGGELER, B., BLÖMER, M., RODENBECK, A. & RUTHER, E. (1992) The metabolic rate of infused L-tryptophan in men: possible clinical implications of the accumulation of circulating tryptophan and tryptophan metabolites. *Psychopharmacologia* **109,** 149-152
LEBELT, D., ZANELLA, A. J. & UNSHELM, J. (1998) Physiological correlates associated with cribbing behaviour in horses: changes in thermal threshold, heart rate, plasma beta-endorphin and serotonin. *Equine Veterinary Journal Supplement* **27,** 21-27
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 Science* **12,** 57-64
LUCKI, I. (1998) The spectrum of behaviors influenced by serotonin. *Biological Psychiatry* **44,** 151-162
MCGREEVY, P. D., WEBSTER, A. J. F. & NICOL, C. J. (2001) Study of the behaviour, digestive efficiency and gut transit times of crib-biting horses. *Veterinary Record* **148,** 592-596
MAES, M. & MELTZER, H. Y. (1995) The serotonin hypothesis of major depression. In Psychopharmacology: the Fourth Generation of Progress. Eds F. E. Bloom, D. J. Kupfer. Raven Press. pp 933-944
MARKUS, C. R., JONKMAN, L. M., LAMMERS, J. H. C. M., DEUTZ, N. E. P., MESSER, M. H. & RIGTERING, N. (2005) Evening intake of α-lactalbumin increases plasma tryptophan availability and improves morning alertness and brain measures of attention. *American Journal of Clinical Nutrition* **81,** 1026-1033
MENCH, J. A. & SHEA-MOORE, M. M. (1995) Moods, minds and molecules: the neurochemistry of social behaviour. *Applied Animal Behaviour Science* **44,** 99-118
MUELLER, R. K., COOPER, S. R., TOPLIFF, D. R., FREEMAN, D. W., MACALLISTER, C. & CARTER, S. D. (2001) Effect of dietary cation-anion difference on acid-base status and energy digestibility in sedentary horses fed varying levels and types of starch. *Journal of Equine Veterinary Science* **21,** 498-502
NADEUA, J. A., ANDREWS, F. M., MATHEW, A. G., ARGENZIO, R. A., BLACKFORD, J. T., SOHTELL, M. & SAXTON, A. M. (2000) Evaluation of diet as a cause of gastric ulcers in horses. *American Journal of Veterinary Research* **61,** 784-790
NICOL, C. J., WATERS, A. J., WILSON, A. D., DAVIDSON, H. P. D. & HARRIS, P. A. (2002) Study of crib-biting and gastric inflammation and ulceration in young horses. *Veterinary Record* **151,** 658-662
ORTIZ, J., ARTIGAS, F. & GELPÍ, E. (1988) Serotonergic status in human blood. *Life Sciences* **43,** 983-990
PICCIONE, G., ASSENZA, A., FAZIO, F., PERCIPALLE, M. & CAOLA, G. (2005) Central fatigue and nyctohemeral change of serum tryptophan and serotonin in the athletic horse. *Journal of Circadian Rhythms* **28,** 6
PICCIONE, G., CAOLA, G. & REFINETTI, R. (2004) Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* **21,** 571-589
RAHMAN, M. K., NAGATSU, T., SAKURAI, T., HORI, S., ABE, M. & MATSUDA, M. (1982) Effect of pyridoxal phosphate deficiency on aromatic L-amino acid decarboxylase activity with L-DOPA and L-5-hydroxytryptophan as substrates in rats. *Japanese Journal of Pharmacology* **32,** 803-811
RUDNICK, G., KIRK, K. L., FISHKES, H. & SCHULDINER, S. (1989) Zwitterionic and aniomic forms of a serotonin analog as transport substrates. *Journal of Biological Chemistry* **264,** 14865-14868
SARRIAS, M. J., CABRÉ, P., MARTÍNEZ, E. & ARTIGAS, F. (1990) Relationship between serotoninergic measures in blood and cerebrospinal fluid simultaneously obtained in humans. *Journal of Neurochemistry* **54,** 783-786
SHUSTER, L. & DODMAN, N. H. (1998) Basic mechanism of compulsive and self-injurous behaviour. In Psychopharmacology of Animal Behaviour Disorders. Eds N. H. Dodman, L. Shuster, M. A. Malden. Blackwell Science. pp 185-202
SOLOMON, M. B. & HERMAN, J. P. (2009) Sex differences in psychopathology: of gonads, adrenals and mental illness. *Physiology and Behaviour* **97,** 250-258
TINKER, M. K., WHITE, N. A., LESSARD, P., THATCHER, C. D., PELZER, K. D., DAVIS, B. & CARMEL, D. K. (1997) Prospective study of equine colic risk factors. *Equine Veterinary Journal* **29,** 454-458
UEHARA, T., SUMIYOSHI, T., ITOH, H. & KURATA, K. (2008) Lactate production and neurotransmitters; evidence from microdialysis studies. *Pharmacology, Biochemistry and Behaviour* **90,** 273-281
VERVUERT, I., COENEN, M. & WATERMÜLDER, E. (2005) Metabolic responses to oral tryptophan supplementation before exercise in horses. *Journal of Animal Physiology and Animal Nutrition* **89,** 140-145
WALSH, A. E., OLDMAN, A. D., FRANKLIN, M., FAIRBURN, C. G. & COWEN, P. J. (1995) Dieting decreases plasma tryptophan and increases the prolactin response to d-fenfluramine in women but not men. *Journal of Affective Disorders* **21,** 89-97
WALTHIER, D. J., PETER, J. U., BASHAMMAKH, S., HÖRTNAGL, H., VOITS, M., FINK, H. & BADER, M. (2003) Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* **299,** 76
WILSON, A. D., BADNELL-WATERS, A. J., BICE, R., KELLAND, A., HARRIS, P. A. & NICOL, C. J. (2007) The effects of diet on blood glucose, insulin, gastrin and the serum tryptophan: large neutral amino acid ratio in foals. *Veterinary Journal* **174,** 139-146
WINBERG, S., ØVERLI, Ø. & LEPAGE, O. (2001) Suppression of aggression in rainbow trout (*Oncorhynchus mykiss*) by dietary L-tryptophan. *Journal of Experimental Biology* **204,** 3867-3876
ZHANG, X., BEAULIEU, J. M., SOTNIKOVA, T. D., GAINETDINOV, R. R. & CARON, M. G. (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. *Science* **9,** 217