Abstract: The current experiment examined the effect of switching from a moderate grain diet to a high grain diet, as are typically fed to intensively managed horses, on some behavioural and physiological (oro-caecal transit time [OCTT], digestibility, plasma cortisol concentrations and heart rate) parameters in adult cribbers (n = 5), weavers (n = 6) and non-stereotypic Thoroughbred horses (n = 6). The cribbers and weavers in the study had been known to show stereotypic behaviour for at least 12 months prior to commencement of the study. Switching to a high grain diet reduced explorative behaviour (P < 0.01) but did not affect stereotypic behaviour. Horses took more time to consume the entire high grain ration than the moderate grain ration (P < 0.01), possibly in an attempt to slow the rate of starch delivery to the hindgut. The high grain diet had lower digestibility than the moderate grain diet (P < 0.01). Switching to a high grain diet was accompanied by a reduction in water intake (P < 0.05), though the two diets did not differ significantly in their effects on plasma cortisol concentration, heart rate, or OCTT. Combined, these findings suggest that horses may have altered their feeding habits in response to the change in diet and this change in behaviour could have been an attempt to limit diet induced acid build-up in the hindgut. Future studies on the relationship between the level of grain in the diet and stereotypic behaviour should consider that horses may show feeding changes to different diets which attenuate diet-induced effects on physiology and other behaviour.

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The effects of two different amounts of dietary grain on the digestibility of the diet and behaviour of intensively-managed horses.

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Abstract

The current experiment examined the effect of switching from a moderate grain diet to a high grain diet, as are typically fed to intensively-managed horses, on some behavioural and physiological (oro-caecal transit time [OCTT], digestibility, plasma cortisol concentrations and heart rate) parameters in adult cribbers (n=5), weavers (n=6) and non-stereotypic Thoroughbred horses (n=6). The cribbers and weavers in the study had been known to show stereotypic behaviour for at least 12 months prior to commencement of the study. Switching to a high grain diet reduced explorative behaviour (P<0.01) but did not affect stereotypic behaviour. Horses took more time to consume the entire high grain ration than the moderate grain ration (P<0.01), possibly in an attempt to slow the rate of starch delivery to the hindgut. The high grain diet had lower digestibility than the moderate grain diet (P<0.01). Switching to a high grain diet was accompanied by a reduction in water intake (P<0.05), though the two diets did not differ significantly in their effects on plasma cortisol concentration, heart rate, or OCCT. Combined these findings suggest that horses may have altered their feeding habits in response to the change in diet and this change in behaviour could have been an attempt to limit diet-induced acid build up in the hindgut. Future studies on the relationship between the level of grain in the diet and stereotypic behaviour should consider that horses may show feeding changes to different diets which attenuate diet-induced effects on physiology and other behaviour.
1. Introduction

Grains are often a major part of the diet of intensively-managed horses, resulting in a proportion of undigested grain passing through to the hindgut where it is rapidly fermented. The consequences of this include an over-production of acid in the hindgut and a resultant drop in caecal pH (Rowe et al., 2001). By way of a further example, Cuddeford (1999) reported that starch intakes of 2.4g /kg bodyweight per meal significantly depressed caecal pH due to fermentative acidosis. For these reasons, Meyer et al. (1995) cautioned that although the capacity of the equine foregut to digest starch appears to be greater than 2g /kg bodyweight per meal, not more than this amount should be provided to horses.

Willard et al. (1977), Johnson et al. (1998) and Zeyner et al. (2004) suggest that decreased caecal pH, caused by the digestion of concentrated diets, may increase the horse’s motivation to chew wood, eat bedding, practise coprophagy and exhibit nervousness, restlessness and aggressive behaviour. Although a link between caecal pH and, in particular, oral behaviour is implied by these findings, the mechanism by which a reduction in hindgut pH may influence behaviour is not fully understood. One possibility is that abnormally acidic conditions in the hindgut may cause discomfort to the horse, resulting in expression of anxiety-related oral behaviours (Johnson et al., 1998).
Additionally, many authors have suggested low forage feeding practices as a factor in the development and performance of stereotopies in horses (e.g. Kiley-Worthington, 1987). Gillham et al. (1994), McGreevy et al. (1995), Redbo et al. (1998), Bachmann et al. (2003) and Zeyner et al. (2004) reported a significantly greater risk of abnormal oral or locomotory behaviours in horses fed concentrated, low roughage diets. Additionally, foals weaned onto a concentrated diet have been shown to have a four-fold increase in the risk of development of crib-biting, when compared with foals weaned onto a high-roughage diet (Waters et al., 2002). Supplying a variety of concentrates and forages has also been reported to reduce stereotypic behaviour in adult horses (Goodwin et al., 2002; Goodwin et al., 2005). These data suggest that the origins and onset of crib-biting and perhaps locomotory stereotypies in horses may be strongly related to diet.

The current study examined the effects on behaviour, physiology and digestibility of changing from a diet containing a moderate level of grain to a diet containing a high level of grain (levels commonly fed to intensively managed horses). Different behavioural and physiological attributes of stereotypic versus non-stereotypic horses have already been reported in the subjects of this study (by Clegg et al., 2008), and it was expected that changing to a higher starch diet would affect cribber, weavers and control horses differently. Our hypothesis was that changing to a high grain diet would lead to
increased cribbing in cribbers, and other behavioural and physiological changes in weavers and control horses indicative of discomfort and/or frustration.

2. **Methods**

2.1 **Horses**

Seventeen adult Thoroughbred geldings, ranging from 3-18 years were used in this study. Case histories and initial observations on stereotypic behaviour were used to classify “cribbers” (N=5), “weavers” (N=6) and non-stereotypic control horses (N=6). Initial bodyweights ranged from 464 kg to 630 kg. Mean commencement weights (± se) for the cribbers, weavers and non-stereotypic control horses were not significantly different (541.2 ± 27.0 kg, 503.3 ± 16.1 kg and 539.0 ± 19.4 kg, respectively). To minimise the effect of different management practices prior to the start of the experiment, horses arrived a minimum of seven days prior to commencement of the experiment and received identical treatment throughout this acclimatising period.

2.2 **Housing**

Housing was as described by Clegg et al. (2008). Briefly, each stable had a floor area of 4 × 4 metres and horses were able to make olfactory, visual and tactile contact with neighbouring horses on three sides. All horizontal surfaces of the stables were made of metal and, as an alternative to cribbing on metal, a wooden bar (approximately 60cm long, 10cm × 8cm) was fixed to a side wall
of each stable to facilitate and focus cribbing. A standard video surveillance camera was mounted above each stable and linked to a sequential switcher and time-lapse video recorder. Fluorescent lighting remained on throughout the trials, allowing good visibility at night, but horses were returned to natural photoperiods after the experiment.

2.3 Experimental Design

Experimental work was carried out as two identical 17-week periods, August to December 1999 and February to June 2001 with 10 horses being tested in the first period and seven horses tested in the second. Week 0 was an acclimatisation week, followed by treatment weeks 1 - 16. Horses spent 22h per day in their stables and were given 2h free exercise in a paddock each afternoon (commencing at 13:30h), during which no data were collected.

Two feeding treatments providing maintenance rations were given to each horse for 8 weeks. During weeks 1 - 8, horses were fed a moderate grain diet consisting of 35% oat grain, 27% lucerne chaff/hay and 38% wheaten chaff (approximately 2g starch /kg bodyweight). During weeks 9-16, horses were fed a high grain diet containing 50% oat grain, 1% lucerne chaff/hay and 49% wheaten chaff (approximately 3g of starch /kg bodyweight). Both diets provided an energy level of 2.4Mcal digestible energy (DE)/kg dry matter, and met the daily feed weight of 1.7% of estimated ideal bodyweight. Feed was delivered in two equal rations at 10:00h and 17:00h each day. These rations and
feeding schedules provided the horses with a level of starch relatively typical in
the diets of racehorses or other intensively-managed horses.

2.4 Behaviour Measurements
Horses were videoed during the 22h per day that they were in the stables. A
sequential switcher was attached to the camera system, filming each horse for
30s in every five-minute period. The video recordings were later viewed and
the presence or absence of nine behavioural elements, described by Clegg et al.
(2008), recorded for each 30s period.

2.5 Physiology Measurements
Venous blood samples for cortisol measurement were taken from each horse
every second morning at 09:00h of the last week of each feeding period. Plasma
cortisol assays were carried out using cortisol RIA assay kits (Active Cortisol
RIA DSL – 2100, Diagnostic Systems Laboratory, Texas USA), and a mean
from the three recordings was obtained for each horse. Mean daily heart rate
(excluding turn-out time) was measured for each horse throughout the duration
of the trial, using heart rate monitors sampling at every 15s (Polar Accurex
plus™, Polar Electro™, Finland) attached to regular lunge rollers.

2.6 Oro-caecal transit time (OCTT)
OCTT was measured once on either the last or second to last day of each
feeding period using the method developed for use in horses by McGreevy and
Nicol (1998). After a baseline pre-feeding blood sample was withdrawn, horses were orally dosed with a sulphasalazine (Salazopyrin, Kabi Pharmacia Ltd, Milton Keynes, UK) at a rate of 50mg/kg, followed immediately by delivery of the morning feed ration. Sequential venous samples were withdrawn from each horse every 30min for the following seven hours via indwelling catheters. Sulphapyridine was detected by High Performance Liquid Chromatography (HPLC), as described by McGreevy and Nicol (1998). The threshold of first appearance was set at the point where the ratio of sulphapyridine to internal standard (sulphamethazine) reached 0.02.

2.7 Digestibility

N-alkane concentrations in feed and faeces were measured to determine digestibility, using the method described by Mayes et al. (1986) and modified by Dove (1992). Horses were accustomed to wearing faecal collection harnesses (Equisan™, Australia) during the seven day acclimatisation period, and wore these throughout the trial (excluding turn-out time).

Grab-samples of faeces were removed from the faecal collection harness every 6h for a period of 48h during weeks 4, 8, 12 and 16. Feed and faecal samples were subsequently frozen at -4ºC, then oven dried to a constant weight at 60ºC prior to grinding through a 1mm sieve using a Cyclone™ sample mill (UDY Corporation, Fort Collins, Colorado, USA).
Alkane concentrations were measured using the alkane extraction method described by Dove (1992). Values for C31 were chosen for use in the analysis, as the concentration of this alkane is high in feed and faeces and had the greatest correlation between feed values and faecal values. Raw data were corrected for faecal recovery, using the recovery value of 94.0 ± 2.8% (O’Keefe and McMeniman, 1998).

2.9 Water intake

Each afternoon, the volume of water required to refill each 60L bucket was recorded to provide a measure of the volume of water consumed per day.

2.9 Animal Ethics considerations

The study was conducted with the approval of the Charles Sturt University Animal Care and Ethics Committee (ACEC). Permission to use a randomised treatment design was not granted by the ACEC, as it was deemed unethical to commence horses on a high grain diet without first acclimatising them to a moderate grain diet, so the design adopted investigates the effects of changing from a moderate to a high grain diet. Subsequently, some effects of time and order of treatments cannot be entirely discounted and the authors acknowledge that the statistical analysis undertaken in this study does not categorically eliminate these possible effects.
Additionally, two hours of turn-out time was a condition of ACEC approval and this affected the extent to which gut function (notably digestibility) could be studied, as exact additional intake of fibre at pasture and total faecal output could not be determined.

2.10 Statistical approach

With sequential treatments, as discussed above, statistical results rest on the assumption that there was no time effect. Behaviour, water intake, plasma cortisol concentrations and heart rate were analysed in an ANOVA with group (weavers, cribbers and control horses) as a between-subject factor and diet (35% grain and 50% grain) as a within-subject factor.

OCCT and digestibility were measured in five cribbers and five control horses as digestive processes have been implicated in the development and performance of cribbing behaviour (Bachmann et al., 2003; McGreevy et al., 1995), and were analysed in a repeated measures 2x2 ANOVA. Additionally, faecal pH could not be measured because samples were destroyed due to freezer malfunction.

All these variables met assumptions for parametric analysis, except for stereotypic behaviour and the time taken to consume the food rations which were (Ln +1) transformed in order to produce a normal distribution. Rubbing
was observed only infrequently and was not analysed. Software by SPSS (Version 14.0, SPSS Inc., Chicago, USA) was used to perform the analysis.

3. Results

Horses took less time to consume the entire ration of moderate (35%) grain diet than to consume the ration of high (50%) grain diet ($F_{(1,14)}= 9.36$, $P=0.008$) (Table 1). There was also a tendency for control horses to consume their rations in less time than cribber and weaver horses ($F_{(2,14)}= 3.4$, $P=0.06$) (Table 1) with no significant interaction between diet and horse group in the time taken to consume the ration ($F_{(1,14)}= 0.97$, $P=0.41$). The eating frequency (i.e. observed with head in the food bin), however, did not differ significantly for diet and horse group (Table 2). Horses explored their stables less when fed the 50% grain diet than the 35% grain diet (Table 2).

No other significant differences in behaviour were found between the cribbers, weavers and control horses (all $P>0.2$) or in the interaction between horse group and grain diet (all $P>0.1$). Contrary to expectation, diet had no effect on the frequency of stereotyped behaviour in cribber and weaver horses (82 and 110 events per day respectively, ANOVA on transformed data $F_{(1,14)}= 1.24$, $P=0.29$). Horses tended to defecate less frequently on the 50% grain diet than the 35% grain diet (Table 2). Water intake was also lower when fed the 50% grain diet than the 35% grain diet (Table 2), though drinking time was not
significantly affected by diet (Table 2). No other significant effects of diet on behaviour were observed (Table 2).

The percentage digestibility of the 35% medium grain diet was greater than the digestibility of the 50% grain diet (Table 3). Diet had no significant effects on plasma cortisol concentration, mean heart rate and OCCT (Table 3). No significant effect of horse group or horse group/diet interaction was found on plasma cortisol concentration, heart rate, OCCT and digestibility (Table 3).

4. Discussion

In summary, changing from a moderate grain diet to a high grain diet led to a clear effect on feeding behaviour, with horses increasing the time taken to consume the high grain ration and drinking less, which would alter the rate of passage of food into the hindgut. When fed the high grain diet, horses explored the stables less often and tended to defecate less often than when fed the moderate grain diet. No evidence was found that a change to high (50%) grain diet increased cribbing or weaving compared with an initial moderate 35% grain diet. Unfortunately, faecal pH samples were destroyed due to freezer malfunction, so we cannot be certain if the high grain diet resulted in a lower caecal pH level than the moderate grain diet as expected (e.g. Zeyner et al., 2004). The high grain diet had lower digestibility than the moderate grain diet,
possibly indicative of a disturbance to microbial fermentation. Changing the diet had no effect on plasma cortisol concentration, heart rate or OCCT.

Slowing down the rate of intake when on the high grain diet could be an attempt to slow down the rate of starch delivery to the caecum and limit the increase in lactic acid. The lower digestibility of the high grain diet is probably due to associative effects of hay and grain (Karlsson and Rundgren, 2000; Kienzle, 1994) and suggests some perturbation of microbial fermentation on the high grain diet could have occurred due to acidic conditions. Additionally, our findings that horses performed less exploratory behaviour when on the high grain diet than on the moderate grain diet may indicate a reduced motivation to eat (i.e. satiety; Ninomiya et al., 2007). It should be noted that an apparent reduction in the motivation to eat could also arise if the horses were experiencing some level of hindgut discomfort. In foals, a high starch diet increases distress after weaning and decreases explorative behaviour compared to foals fed a fat and fibre diet (Nicol et al., 2005). Nicol et al.’s (2005) findings were based on various behavioural tests conducted about 7 months after commencing feeding on the two diets. Our findings extend those of Nicol et al. (2005) by suggesting that a high grain diet affects explorative behaviour relatively quickly (within the first week of a diet change), perhaps due to increased gut discomfort, and that the link between exploration and diet is also seen in older horses.
The absence of an effect of increased grain on frequency of stereotypic behaviour contrasts with the prediction from the literature that high grain levels lead to an increase in cribbing (Bachmann et al., 2003; Gillham et al., 1994; McGreevy et al., 1995; Redbo et al., 1998; Waters et al., 2002; Zeyner et al., 2004). As discussed above, one possibility for this lack of effect is that the changes in feeding behaviour associated with changing to a high grain diet is attenuated by other effects on behaviour and physiology. Alternatively, an effect of high grain levels on stereotypies may have been evident if compared with a roughage-only, fat-based diet (Zeyner et al., 2002) or processed grain diet, such as extruded pellets, that have undergone some destruction of starch structure leading to an improvement of starch digestibility in the small intestine and prevention of excessive hindgut acidosis (Meyer et al., 1995). However, in a practical situation, particularly in racehorses or other intensively-managed horses, a moderate to high concentrate level is often required to provide the horses with sufficient energy for their levels of exercise (Zeyner et al., 2004). Another possible explanation is that stereotypies in horses have a tendency to become “fixed” with time, as is likely in our sample, and tend to be resistant to any attempts to reduce their frequency by management interventions (Cooper and Albentosa, 2005).

5. Conclusion
We conclude that the changes in time taken to consume the ration represent behavioural changes by the horse to the challenges of increased grain in the diet
which are sufficient to compensate for any diet-induced effects on stereotypic behaviour. Future studies on the relationship between the level of grain in the diet and stereotypic behaviour in adult horses should provide diets with a greater variation in starch content than those applied here, to remove the possibility of horses being able to show behavioural changes to the diets which mask any diet-induced effects on behaviour.

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