**A preliminary study of grazing intakes of ponies with and without a history of laminitis**

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Short title: Grazing intakes of ponies with and without laminitis

## Abstract

One possible factor involved in the aetiology of laminitis is grazing intake. Whilst some studies have looked at grazing intake in healthy animals, there has been little comparison made between animals with and without a history of laminitis. The aim of this study was to compare grazing intake between health animals and those with a known history of laminitis. Sixteen mature grass-kept (maintained at grass 24 hours a day) native breed ponies from World Horse Welfare in Norfolk were used in the study, which was conducted in the month of July for a period of 12 days. All animals were grazed under identical conditions. Grazing areas were of that suitable for the management of animals predisposed to laminitis (for ethical reasons) and therefore herbage mass was low (Yield: 124 kg dry matter/ha; sward height of 1-2 cm). Faecal samples were collected from 8 clinically normal horses (NOR) and from 8 that were predisposed to laminitis (LAM) in July 2005. Grazing intake was measured using the alkane technique. Dry matter intakes (DMI) per kilogram bodyweight were low in both groups of animals: 1.32 ± 0.31 percent versus 1.62 ± 0.74 percent for NOR and LAM, respectively. There was no difference in DMIbetween the two groups of ponies (4.43 versus 4.25 kg/day for NOR and LAM, respectively). Mean DMI per kilogram bodyweight per day were 1.32 and 1.62 for NOR and LAM, respectively (20 percent difference).There was a greater variability of DMI within the LAM group with intakes ranging from 0.81 to 2.36 percent bodyweight. The low DMI values were attributed to the overgrazed nature of the pasture used in this study, which was unavoidable due to the welfare issues associated with grazing overweight, laminitis-prone horses on good grazing pasture. Further work is required with a larger study population grazing pastures with greater herbage mass.

**Key words:** equine, laminitis, grazing intake

**Implications:**

Pasture-induced laminitis is thought to be due to excessive ingestion of grass. What is unknown is why only certain animals develop laminitis even when grazed on identical pastures, which may be due to some animals eating more than others. This study investigated grazing intakes between ponies with (LAM) and without (NOR) a history of laminitis. Results showed no difference in intakes between the LAM and NOR groups. However, the LAM ponies ate 20 percent more grass overall compared to the NOR group.This implies that more research is needed with larger groups of animals to determine if differences in intakes exist between LAM and NOR groups and if this may be a predisposing factor in the onset of laminitis.

## Introduction

Laminitis has widespread implications for equine welfare; it has the highest death rate of any orthopaedic condition and is the second largest killer of horses in the UK next to colic. It is also extremely common; in a survey involving 113000 horses in the UK a prevalence of 7.1% was noted (Hinkley and Henderson, 1996). Clinical laminitis represents the end result of a systemic condition that can have many predisposing factors as outlined by Trieber (2006).The pathogenesis of acute laminitis and in particular the relationship between hindgut disturbances and the pathological mechanisms in the digit are crucial to the understanding of the pathogenesis of this disease. Although there are also many other potential factors that may contribute to this condition, pasture-induced laminitis appears to be the most common aetiology in the UK (Hinkley and Henderson, 1996). Moreover, the incidence of laminitis in the US is reported to be 2 percent rising to around 5 percent in the spring and summer (Longland and Byrd, 2006). As its name suggests, this is thought to be associated with excessive ingestion of pasture and/or abrupt change in pasture NSC, and studies in the UK suggest an increased prevalence of laminitis during periods of rapid grass growth. Grass storage carbohydrates (water soluble carbohydrates) have been implicated in the onset of pasture-induced laminitis (Longland et al., 1999). However, one intriguing area is why only certain individuals appear to be predisposed to laminitis, even when grazing identical pastures? Several schools of thought exist, including differences in individual susceptibility at the level of the large intestine in the ability of this organ to buffer changes in pH due to lactic acid production, microbial populations, genotypic factors and grazing intake to name but a few (Bailey et al., 2004). Intakes of pasture are reported to range from 1.5 percent to 3.3 percent of bodyweight (BW) per day (Holland et al., 2000;

McMeniman, 2000), indicating a large variation in grazing intakes. However, despite the prevalence of pasture-associated laminitis and the links between intakes and onset of laminitis in some individuals, there have been no studies investigating whether differences in grazing intakes between individuals may affect susceptibility to laminitis. Consequently, the aim of this study was to measure grazing intakes in ponies with and without a history of laminitis, with the hypothesis that animals with a history of laminitis may have greater intakes than those without.

## Materials and methods

*Animals and management*

Sixteen mature grass-kept (maintained at grass 24 hours a day) ponies from World Horse Welfare in Norfolk, United Kingdom were used in the study, which was conducted in the month of July 2005 for a period of 12 days. Eight mares and eight geldings were used in the study, split into two groups of ponies, 8 clinically normal (NOR) and 8 that had a history of laminitis (LAM). There was an equal distribution of mares and geldings in each group. Ponies with a history of laminitis were included in the study if they were diagnosed with acute laminitis 3 or more times during the preceding three years. Bodyweights at the start of the study averaged 308 ± 92 kg, with body condition scores averaging 3 ± 1 on the 0 – 5 scale. Animals were weighed at 0900 hrs on days 1, 4, 8 and 12 and condition scored on days 1 and 12 (Carroll and Huntington, 1998). All animals were grazed under identical conditions in the same paddock. Grazing areas were of that suitable for the management of animals predisposed to laminitis (for ethical reasons), with low herbage mass and small paddock sizes (0.3 acres per pony). Grazing intake was measured according to the techniques described by Dove and Mayes (1991).

*Marker preparation and administration*

Ponies were hand fed a bite-sized Weetabix® (WB: Weetabix Ltd, Kettering, UK) labelled with $C_{32}$ alkane (Fisher Scientific, Loughborough, UK: 10162190) 3 times per day for a period of 12 days. The alkane-labelled WB was prepared in a fume cupboard. 38 g of C32 was dissolved in 380 ml of heptane using a hotplate stirrer on low heat. The resultant solution contained a concentration of 100 mg of $C_{32}$ per ml of heptane and 10 ml of this was added to each WB. The WB then remained in the fume cupboard overnight at ambient temperature to allow for the absorption of the $C_{32}$/heptane solution before being placed into a force-draught oven at 60 $^{o}$C for 16 hours. Prior to removal the temperature was increased to 90 oC for one hour to ensure the $C_{32}$ was fully absorbed. A sub-sample of 5 alkane-labelled WB was retained for laboratory analysis to determine $C_{32}$ dose rate.

*Sward Sampling*

Quadrat samples (900 $cm_2$) were taken to determine the herbage mass of the field on days 5, 7, 9 and 12 of the study. Six herbage samples were taken at random in a large "W" shape across the whole field with grass cut as close to the soil as possible without any visible contamination of the sample. Sward height was determined using a plate meter (F100 Plate Meter, AgriSupplyServices, UK).

Herbage sampling

Herbage sampling began on day 5 of the study and continued to day 12. Samples were taken twice daily at 10 am and 3 pm to 4 pm depending on the grazing activity of the horses. A quadrat sample (900 $cm_2$) was taken of the grass each horse was eating by placing the quadrat as close as possible to where each horse was grazing.

Samples were weighed and then dried at 60 $^{o}$C until constant weigh and ground (to pass through a 1mm dry mesh screen) prior to alkane analyses.

*Faecal sampling*

Faecal sampling for alkane analyses occurred during the last 5 days of the study (days 7 to 12). One complete faecal deposit was collected per horse each day, weighed and a 250 g sub-sample taken, dried at 60 $^{o}$C to constant weight and ground (to pass through a 1mm dry mesh screen) prior to alkane analysis. An additional faecal sample was collected for each horse on day 12 of the study, frozen immediately and transported to the laboratory for determination of microbial populations.

*Alkane analysis*

Herbage and faecal samples were analysed for the natural odd-chain alkane C31 and faecal samples were also analysed for the dosed $C_{32}$ alkane at the Macaulay Institute, Aberdeen, United Kingdom using the method described by Ali et al. (2004). The 5 sub-samples of the WB were also analysed for $C_{32}$ alkane by crushing them and placing them into separate 100 ml glass bottles, which were capped and weighed. Heptane (30 ml) was added to each bottled and these were then re-weighed. The bottles containing the samples were then heated at 55 $^{o}$C for 1 hr in an ultrasonic bath to dissolve the alkane. A sample (0.2 ml) of the warmed solution was then removed from each glass bottle and placed into pre-weighed screw-capped vials. Vials were then capped and re-weighed and 1.3 ml of alkane internal standard (C22 = 0.80131 mg/g and C34 = 0.80166 mg/g) added to each vial and the vial re-weighed. Samples (0.1 ml) were then taken from each vial and placed in separate gas chromatography (GC) vials to which 0.3 ml of dodecane was added. The concentration of C32 was then determined by GC using the conditions described by Ali et al. (2004).

Herbage intake was calculated using the herbage and faecal concentrations of consecutive even- and odd-chain alkanes using the following equation:

Herbage intake (kg DM/day):

$$\frac{Dj \times (Fi/Fj)}{Hi - ((Fi/Fj) \times Hj)}$$

Where:

$D_j$ = dose rate of even chain alkane ($C_{32}$)
$F_j$ = faecal concentration of even chain alkane ($C_{32}$)
$H_j$ = herbage concentration of even chain alkane ($C_{32}$)
$F_i$: = faecal concentration of odd chain alkane ($C_{31}$)
$H_i$: herbage concentration of odd chain alkane ($C_{31}$)

*Statistical analyses*

Data were analysed for significant differences between intakes for the two groups (LAM and NOR) using a t-test in GenStat Release 10.1 (Lawes Agricultural Trust, Harpenden, UK). Pearson's correlation coefficient was used to analyse for any correlation between liveweight/body condition score and dry matter intakes in ponies.

## Results

Herbage mass was low with a yield of 124 kg DM/ha and a sward height of 1-2 cm. There was no difference (P>0.05) in dry matter intakes between the two groups of ponies; 4.43 versus 4.25 kg/day for NOR and LAM, respectively. Intakes per kilogram bodyweight were low in both groups of animals: 1.32 ± 0.31 percent versus 1.62 ± 0.74 percent for NOR and LAM, respectively (Figure 1). Mean intakes per kilogram bodyweight were over 20 percent higher in the LAM group; however, there was a greater variability within the LAM group with intakes ranging from 0.81 to 2.36 percent bodyweight. Bodyweight fluctuated throughout the study; however, there was no change in bodyweight between the start and end of the study period. Body condition score also did not change over the study period There was no correlation (P>0.05) between liveweight/body condition score and intakes in ponies.

## Discussion

The intake values in this current study were lower than that reported previously (Longland et al., 2011). This is most likely attributable to the low herbage mass in the grazing areas used in the current study compared to others, which was unavoidable for welfare reasons due to the LAM group being at higher risk of developing laminitis if grazed on high yielding pasture. Intakes measured during the experimental period ranged from 0.81 to 2.36 percent of bodyweight, with mean values numerically higher by over 20 percent in the LAM group, demonstrating large variation in grazing intakes between animals during the experimental period. This large variation in intakes may be important, since if we taking the upper value of 2.36, intakes of a pasture containing a higcontent of WSC (384 g/kg DM) (Longland and Byrd, 2006) this would result in a 300 kg pony ingesting 2.7 kg of WSC and 2 kg of fructan (based on a high fructan content of 30 percent).

These intakes of WSC and fructan equate to 9 g and 6.6 g of WSC and fructan/kg BW, respectively. These levels of fructan are above the 3 g (Crawford et al., 2007) and 3.75 g (Pollitt et al., 2003) known to elicit the onset of laminitis when given in a single dose. However, whilst fructan has been used to produce an experimental model of laminitis (Pollitt, 2002) there is no evidence to suggest that the ingestion of grass fructan at similar levelselicits the same response (Bailey et al., 2004). There is also a large variation in WSC content of pastures (Hoffman et al., 2001) and thus lower WSC contents (100 g/kg DM) at high intake levels (2.36 percent BW) would result in much lower intakes of WSC (2.6 g/kg BW).

Induction of laminitis under experimental conditions has been conducted using commercially available fructo-oligosaccharies, such as inulin, and it is important to note that there is no evidence to support the use of fructo-oligosaccharides as a suitable model substrate for grass fructan. It is possible that different levels of grass fructan may be required to elicit a similar response seen when inulin is administered Therefore, experimentally-induced laminitis based on model substrates may not be reflective of the naturally occurring disease and thus it is not possible to extrapolate information from such studies to provide recommendations on the amounts of grass fructan required to induce laminitis. Although there has not been a direct link made between the onset of laminitis and the ingestion of pastures containing high levels of non-structural carbohydrates (NSC), it is clear that pasture plays a role in the development of this condition and it is likely that the ingestion of pasture NSC may have a role in eliciting this disease. Therefore, until there is clear evidence that pasture NSC does not elicit the onset of laminitis, it is important to manage animals/pastures in a way that reduces potential intakes of high levels of NSC. Nonetheless, the large variation and fluctuations in WSC and fructan content of pastures reported (Hoffman et al., 2001) makes it difficult to manage animals in a way that ensures limited intakes of pasture NSC.

The fact that, while any horse/pony can succumb to laminitis under experimental conditions using large amounts of NSC administered in a pulse dose and under field conditions only a proportion of animals in a herd may suffer recurrent laminitis whilst others remain unaffected, may be explained by differences in intakes between grazing animals. It would certainly appear from the results of the current study and others (Holland et al., 2000; McMeniman, 2000) that there is a large variability in intakes between animals grazing identical pastures. It is also noteworthy from the current study that intakes may be higher in animals with a history of laminitis, and it is certainly known that obese horses are at greater risk of developing laminitis (Geor and Harris, 2009). It is possible that obese horses may also have higher intakes when grazed under identical conditions to non-obese horses and thus it is conceivable that grazing intakes in certain individuals may not directly elicit the onset on laminitis, but influence obesity, insulin resistance and metabolic syndrome, all of which are associated with an increased risk of laminitis (Geor, 2008). None of the ponies in this current study gained weight or BCS, or developed any health issues; nevertheless, the study period was limited to 12 days and ponies were grazed on pastures with a low herbage mass.

A limitation of the current study was that no chemical analyses were performed on the grazing pasture and thus NSC contents were unknown, but it is likely that this was low given the BW and health status of the ponies during the study. A different picture would likely have emerged if the pasture had a higher herbage mass, with a higher nutrient content and NSC levels, and the study period was extended. The fact that all ponies maintained weight on such limited pasture is noteworthy and suggests that grazing animals on similar pastures may be beneficial for managing horses and ponies that are overweight and/or at risk of developing laminitis. However, further monitoring of changes in BW and BCS over a longer period of time would have been beneficial as 12 days is generally not enough time to see significant changes in BW or BSC (Geor and Harris, 2009).

## Conclusion

It would appear from these results that there was no difference in intakes between NOR and LAM ponies; however values were numerically higher and more variable in the LAM group and thus further work is required with a larger study population and grazing pastures with a greater herbage mass.

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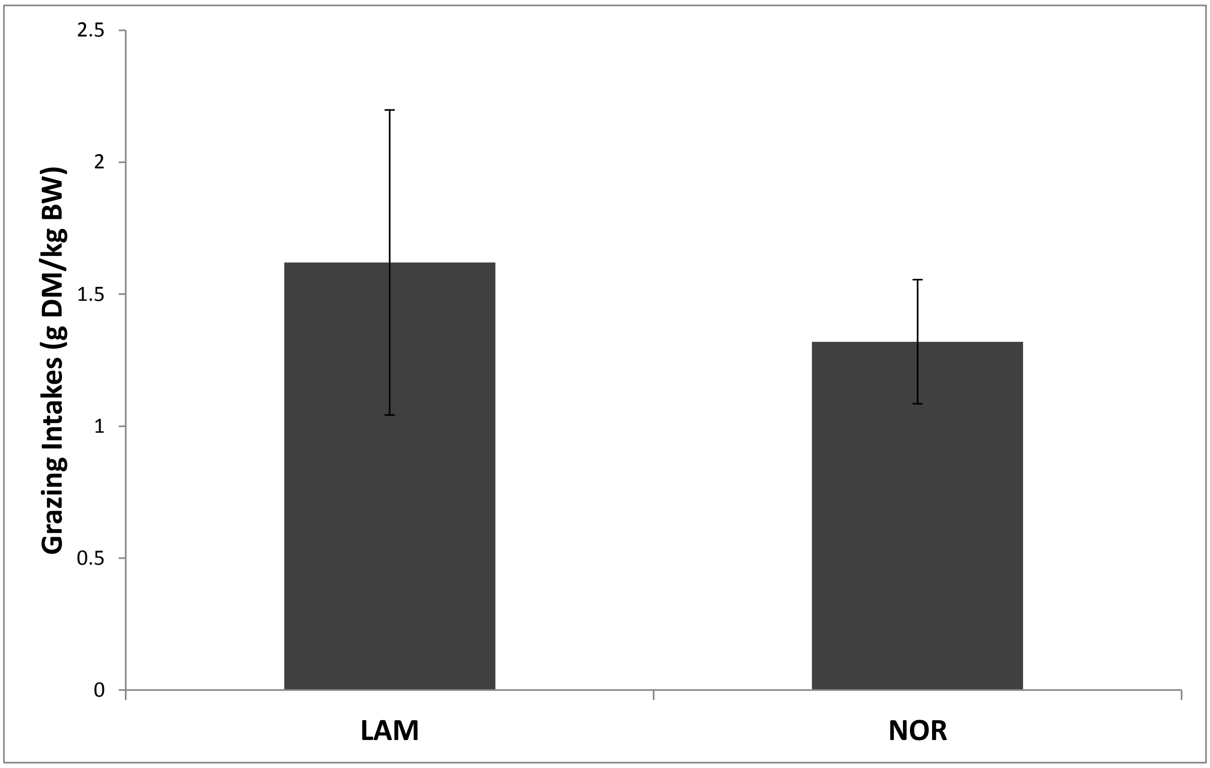



Figure 1: Grazing intakes of ponies with (LAM) and without (NOR) a history of laminitis

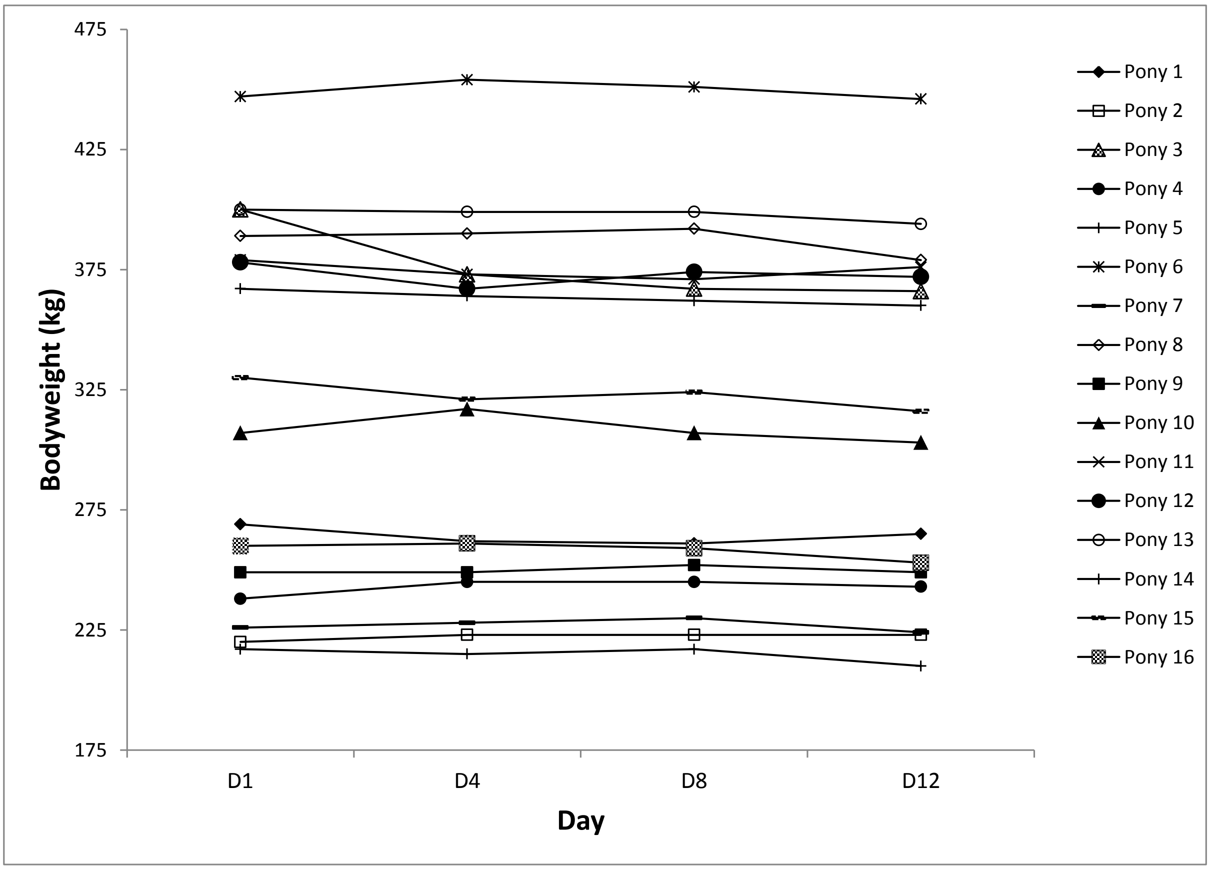
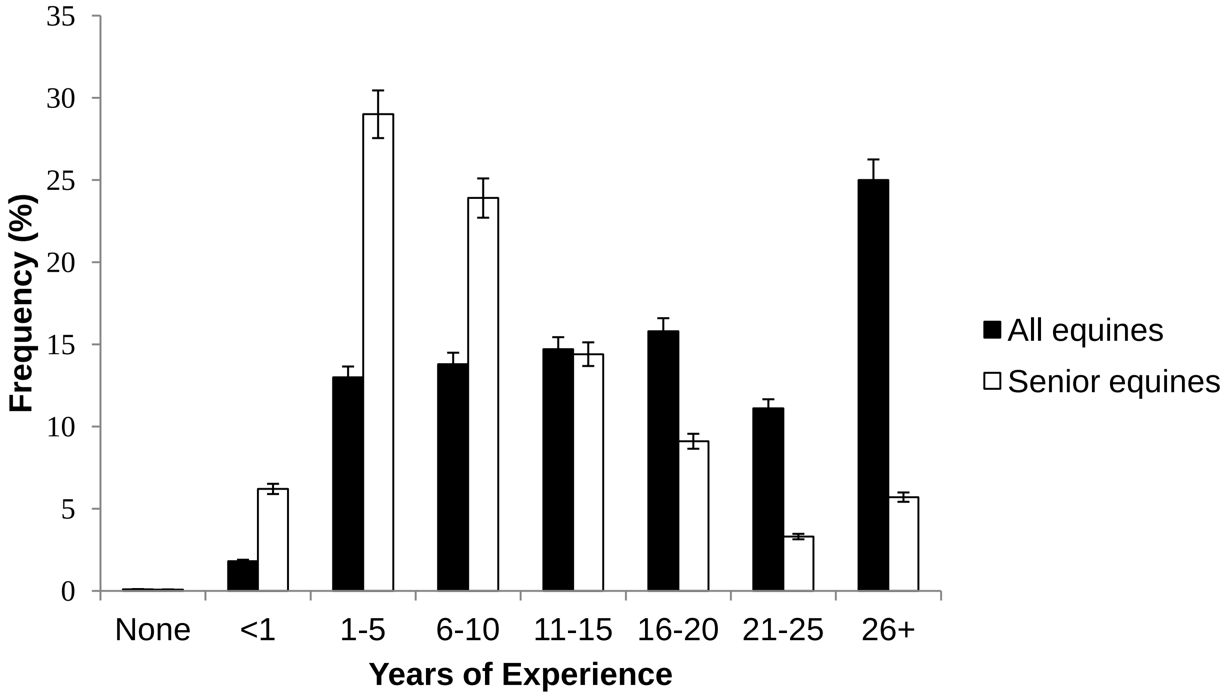


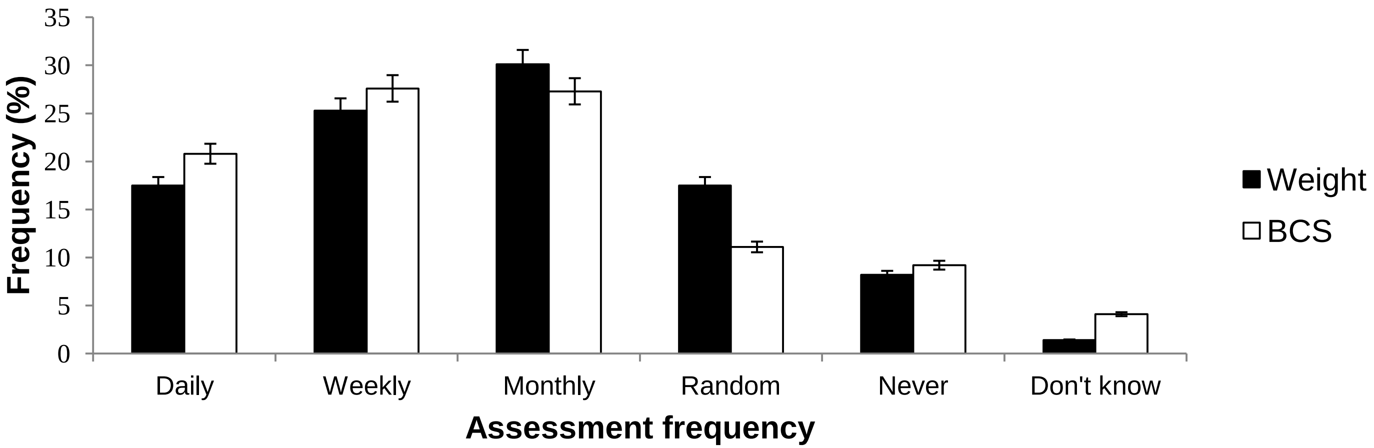

Figure 2: Pony liveweights on days 1, 4, 8 and 12 of grazing

Figure 1



35
30
25
20
15
10
5
0
Frequency (%)
None
<1
1-5
6-10
11-15
16-20
21-25
26+
Years of Experience
All equines
Senior equines

Figure 2



35
30
25
20
15
10
5
0
Frequency (%)
Daily
Weekly
Monthly
Random
Never
Don't know
Assessment frequency
Weight
BCS

Figure 3

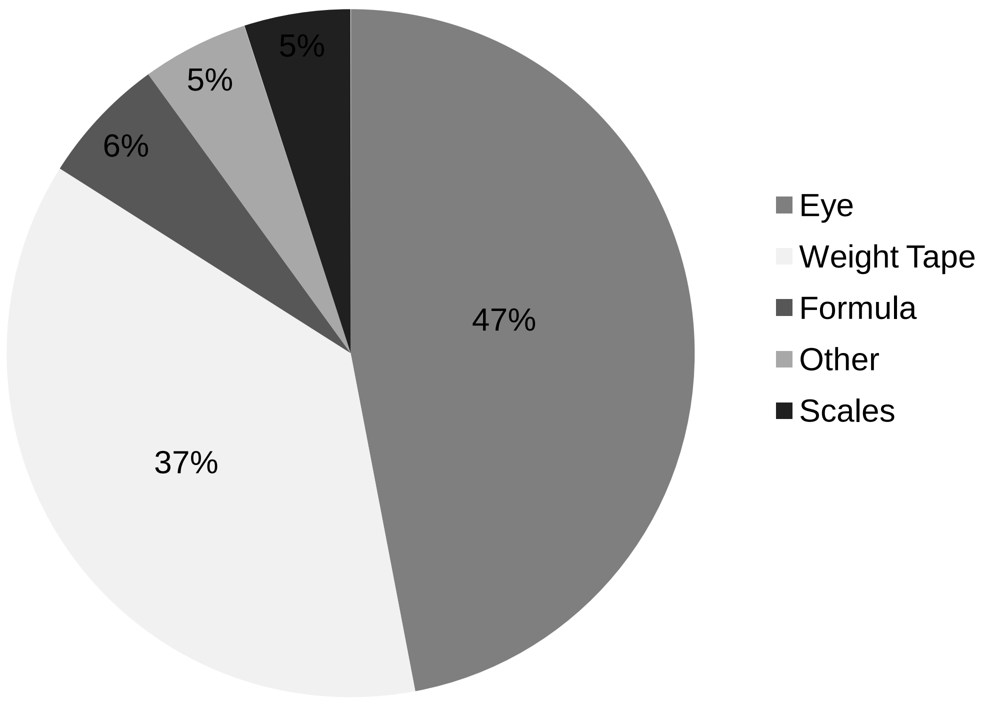


Figure 4

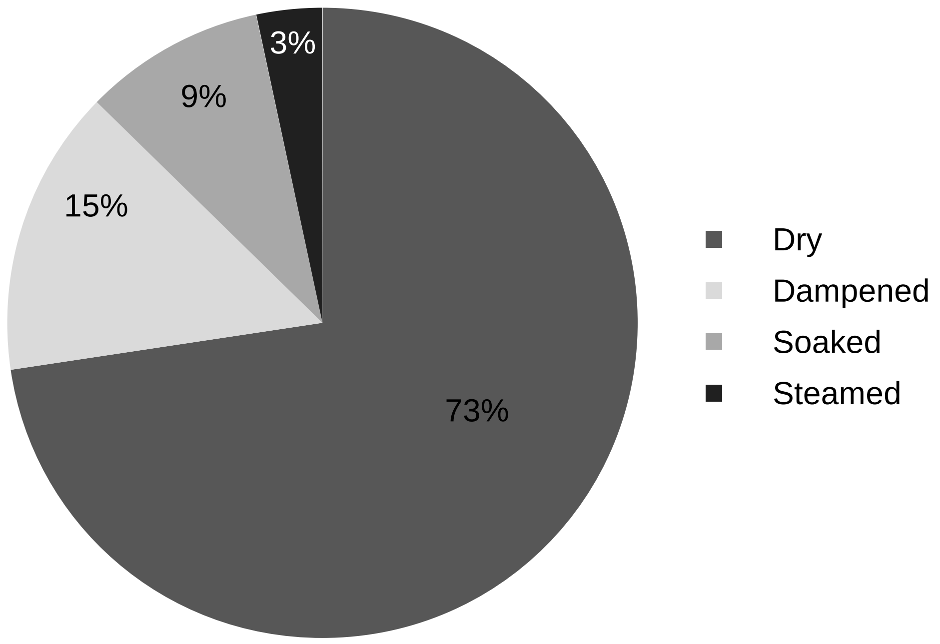
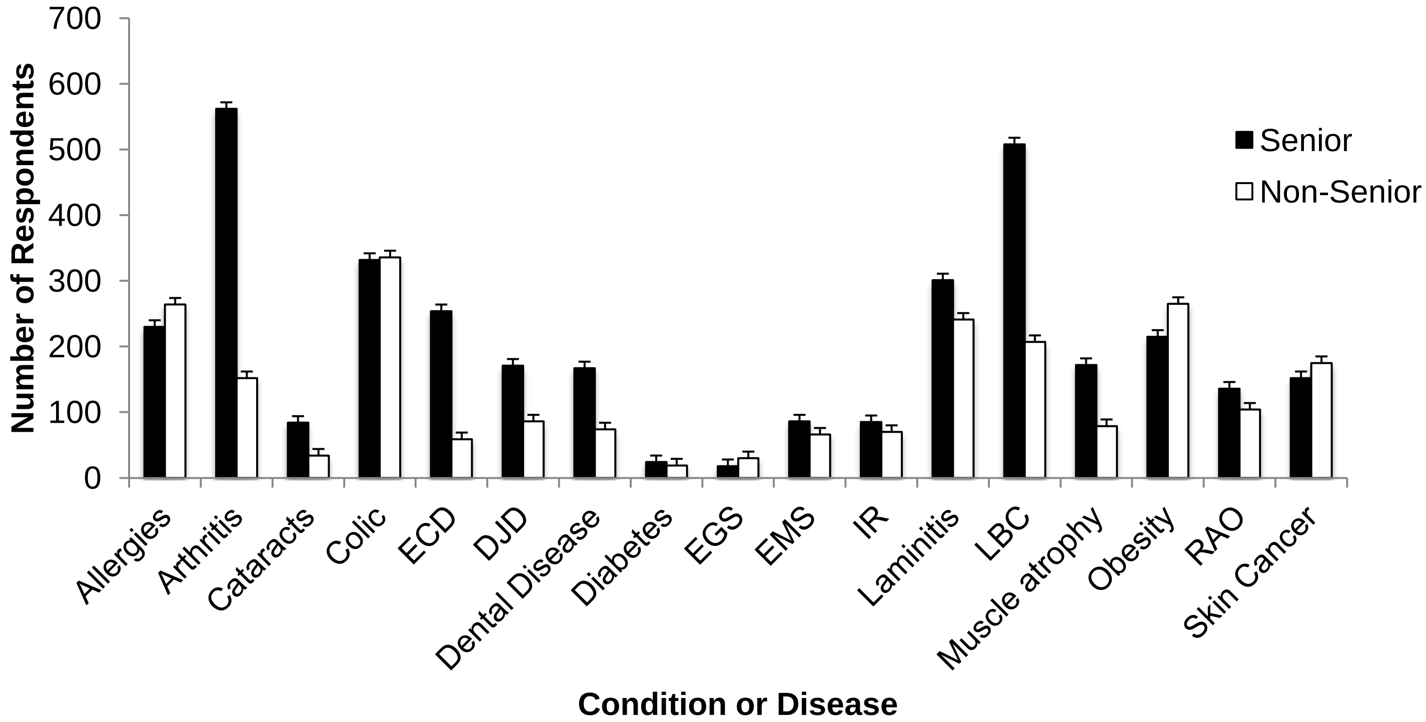
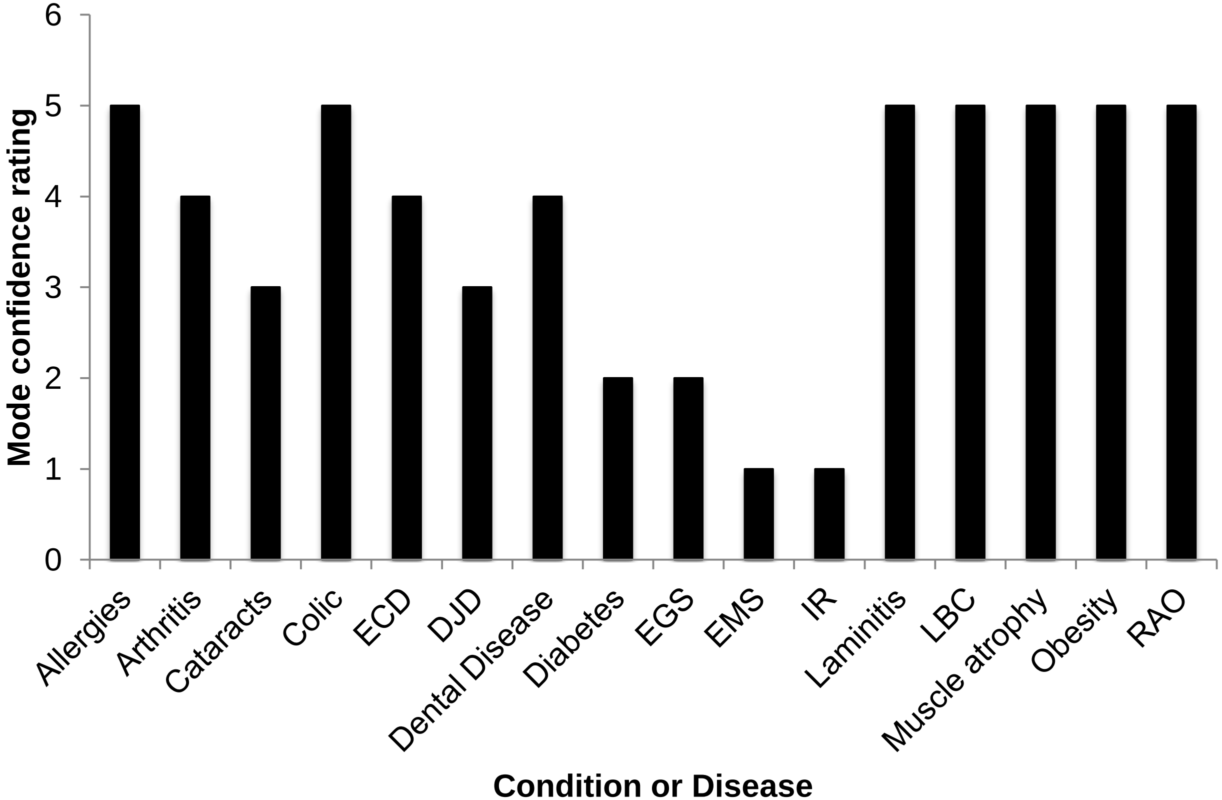


Figure 5



700
600
500
400
300
200
100
0
Number of Respondents
Senior
Non-Senior
Allergies
Arthritis
Cataracts
Colic
ECD
DJD
Dental Disease
Diabetes
EGS
EMS
IR
Laminitis
LBC
Muscle atrophy
Obesity
RAO
Skin Cancer
Condition or Disease

Figure 6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EMS
IR
Laminitis
LBC
Muscle atrophy
Obesity
RAO
Condition or Disease