**Growth rates of Thoroughbred foals and *in vitro* gut health parameters when fed a cereal or an all fibre creep feed.**

M. Moore-Colyer1, Philippa Tuthill1, Isobel Bannister12, Simon Daniels1

1 Royal Agricultural University, Stroud Road, Cirencester, Glos. UK. GL7 6JS

2 Present address: Askham Bryan College, Askham Bryan, Yorkshire, YO23 3FR

Corresponding author: Meriel Moore-Colyer [Meriel.moore-colyer@rau.ac.uk](mailto:Meriel.moore-colyer@rau.ac.uk)

**Declaration of interests**

None

**Open Access**

Yes

**Abstract**

Traditions and the economic advantage when producing big athletic TB yearlings for sale, encourages owners to feed high levels of cereals. Such diets can predispose gastric ulceration and developmental orthopaedic diseases, impacting negatively on future careers. Study aims: 1. Determine if an all fibre creep feed (TMFR) would sustain equal growth rates in TB foals versus a cereal based stud cube (SC); 2. Compare pH and lactate produced by SC and TMFR in *in vitro* foregut and hind gut conditions. Eight TB foals in matched-pairs were fed SC or TMFR for 18 weeks. Weight gain (ADG), height at wither and hip, heart girth and body length were recorded weekly. Similar growth rates were recorded for all measurements. ADG of 1 and 1.1kg/head/d for TMFR and SC respectively. *In vitro* foregut incubation of SC and TMFR at 37oC in pepsin HCl solution for 6 hours, produced higher pH 5.84 (TMFR) compared with 5.33 (SC) (P<0.05). Gas production measured fermentation rate, acidity and lactate from TMFR and SC, incubated with faecal inoculum from the foals on the same diet. Fermentation rates and lag times were equal for both feeds; total gas produced, t 50 and Y50 were greater (P<0.05) for SC. Lactate and pH levels were lower and higher respectively for the TMFR. This study showed that similar growth in TB foals was achieved on the TMFR feed and that potentially better gut health, denoted by higher pH and lower lactate levels could be maintained by fibre compared with cereal feed.

**Key words**

Horses, foals, fibre, growth rates, creep feeding

**1. Introduction**

Producing thoroughbreds for a racing career is a competitive process governed by strong industry trends, traditions and conventions. Yearling sales dates and an early-matured horse for two-year-old racing stimulate breeders to ‘push’ growth rates by feeding high levels of cereal-based concentrates in order to produce bigger, fitter more athletic-looking horses [1]. Pagan and Brown-Douglas [2] noted that at public auction a larger, athletic-looking yearling was more likely to sell above the sale median than a smaller, lighter one. Moreover Luszczynskian and Pieszka [3] demonstrated a significant relationship between growth rate in the first 6 months and racing performance in 2 and 3 year old horses. Thus there are strong incentives for many breeders and consigners to feed to achieve maximum growth rates and this often involves exceeding the maximum recommended starch intake of 1-1.5g/kg body weight per meal [4, 5].

Such regimes can pre-dispose young horses to gastric ulcers [6] and growth-related disorders, particularly developmental orthopaedic diseases (DOD) [7]. Gastric ulcers are common in foals with incidences ranging from 22% in a group of 691 foals in Florida [8] and up to 51% recorded during gastroscopy in the UK [9]. While osteochondritis dissecans (OCD) is a complex multi-factorial condition it has been noted that over feeding concentrates [10, 11, 12] can predispose this condition. Indeed Pagan [10] reported a positive correlation between feed glycaemic index and the incidence of OCD in a group of 218 foals on 6 Kentucky stud farms, and also noted that plasma glucose and insulin 2 h post-feeding were significantly higher in weanlings that had OCD compared with unaffected foals. OCDs are of economic importance to the racing industry. The incidence is high enough in all populations of TBs to stimulate expensive routine radiographic assessment of joints before sales. This is deemed necessary as radiographic changes, particularly of the carpel joint are associated with reduced start rates, racing ability and performance in 2 and 3 year old TB [14,15].

To avoid the above-mentioned conditions, which compromise health, contravene one of the five freedoms of animal welfare and have a negative economic impact on the industry, a better way of feeding that would achieve the desired growth rates, is urgently needed. Such a feeding regime necessitates increasing the level of fibre in the diet. However, many TB breeders are reluctant to do this believing that the lower energy and protein digestibility of fibre feeds reduce growth rates and cause ‘fibre-belly’ in comparison to conventional high starch diets [16,17]. Despite the facts that Saastamoinen and Särkijärvi [18] have reported that weanlings are capable of digesting a high-quality fibre diet, and that reduction of starch and increase in fibre intake aids in the recovery of gastric ulcers in horses, [19] there is still much prejudice against feeding high-fibre diets to young growing TB.

The objectives of this field study conducted on a commercial stud farm were: (1) to measure the intake and growth rates in TB foals when fed a specifically formulated total mixed fibre ration (TMFR) compared with a traditional high-cereal stud cube (SC). (2) To measure stomach pH and hind gut fermentation characteristics of both feeds using simulated foregut and hind gut *in vitro* systems.

**2. Methodology**

*2.1. Trial 1 in vivo intake and growth*

The Animal Ethics Review group of the Royal Agricultural University approved the experimental procedures. The study was conducted as a matched pairs design with 4 Thoroughbred (TB) foals in each group matched according to birth date. Weekly measurements were taken of body weight in kg (BW), heart girth in cm (HG), body length in cm (BL) hip height in cm (HH) and height at withers in cm (WH) for a total of 18 weeks from May to September 2017.

Foals were kept on a commercial stud farm (Bucklands Farm and Stud, Gloucestershire). Each group consisted of 3 fillies and 1 colt, (see Table 1 below). Two foals in each group were 3 months old when they started the trial, one per group were 2 and 1 month old, thus each group had a similar range of ages, sex and breed in an attempt to match growth trajectories across the two groups. Diets were fed from 10th May 2017 until the final weighing point on 4th September 2017, thus all growth measurements were taken 17 times over the 18 week (126 day) trial period.

*2.1.1. Animal Management and Diets*

Foals were with their dams for the first 7 weeks of the trial before weaning. Foals were weaned at approximately six months of age (180 days) in the field. Foals were weaned in their herd, while dams were removed to the next field for a week and then removed completely. The foal groups were kept in one big field (a rye grass and timothy mixed ley) which was divided by electric fencing. Group A, those fed the conventional cereal-based diet of stud cubes, here after referred to as the SC group had a mean LW of 155(±33.5) kg while Group B who were fed the total mixed fibre ration, hereafter referred to as TMFR group, were a little lighter with a mean live weight of 128 (±23.3) kg. This meant that TMFR were 27 kg lighter than SC at the start of the trial (see Table 2 below).

**Table 1**. Foal sex, birthdate and sire for study Groups A fed Stud Cubes (SC) and Group B fed Total Mixed Fibre Ration (TMFR)

|  |  |  |
| --- | --- | --- |
|  | Group A (SC) | Group B (TMFR) |
| Foal  Sex  Birthdate  Sire | A1  Filly  08.02.2017  (Hellvelyn) | B1  Filly 1  12.02.2017  (Casamento) |
| Foal  Sex  Birthdate  Sire | A2  Filly  23.02.2017  (Hot Streak) | B2  Filly  17.02.2017  (Coach House) |
| Foal  Sex  Birthdate  Sire | A3  Filly  03.03.2017  (Coach House) | B3  Colt  01.03.2017  (War Command) |
| Foal  Sex  Birthdate  Sire | A4  Colt  07.04.2017  (Coach House) | B4  Filly 1  3.04.2017  (Coach House) |
|  |  |  |

Both groups were given two feeds per day morning and evening. Group A were fed Sharp Nutrition Stud Cubes (Sharp Nutrition, Newmarket, UK) in a specifically designed creep. The level of feed was set by the stud owner and based on her experience of the previous years when feeding foals SC. At the beginning of the trial foals were fed ½ bag (12.5 kg on an as fed basis) per day which was gradually increased to 1 bag per day (25 kg) for the last month of the trial. Group B were fed a total mixed fibre ration (TMFR) (Eclipse Feeds Ltd, Innishannon, Co Cork, Ireland) by putting TMFR into a specifically designed feeder (Plate 1) morning and evening thus foals had continual access to TMFR. The amount of TMFR fed was based on a previous trial carried out in 2016 at Bucklands Stud when a group of 12 TB foals were fed the TMFR in order to determine palatability, intake level and growth rates on this novel all fibre creep feed. It was during this preliminary field trial that the amount fed and feeding system was developed and used during the current trial. At the beginning of the trial, foals were fed 1 bag (20 kg on an as fed basis) which was gradually increased to 2 bags /day (40kg) for the last month of the trial. By the end of the trial the stud owner wanted to ensure that foals always had access to creep feed, thus the reason why 1 bag of SC and 2 bags of TMFR were fed. Any feed rejections were removed before the fresh feed was put into the feeder in order to keep the feeders free from any possible microbial build up from degrading feed. Feed intakes were calculated from the number of bags fed minus an estimated amount (based on the amount swept into a bucket) of feed refusals collected per day. Nutritional profiles for each diet are detailed in Table 3. The authors in collaboration with Eclipse Feeds Ltd (Innishannon, Ireland) compiled the TMFR, which was specifically formulated as a creep feed to support the early growth rates seen in TB foals. The feed contains high quality fibre, oil and small-intestine digested non-cereal protein sources. The specifically designed feeders were contained within a creep to prevent the dams consuming any of the feed. Water, grass and dam’s milk (until weaning) were freely available to all foals.

**Table 2.** Nutritional content % (on DM basis) of the Sharp Nutrition Stud Cubes and the Total Mixed Fibre ration offered to Group A and Group B foals respectively

|  |  |  |
| --- | --- | --- |
| Nutrient % | Total Mixed Fibre Ration (Eclipse ® ) | Sharp Nutrition Stud Cubes (Sharp Nutrition ltd) |
| DE (MJ/kg) | 14.9 | 13.8 |
| CP | 15.5 | 16 |
| Ash | 6.0 | 9 |
| NDF | 31.3 | 22.2 |
| ADF | 18.5 | 7.61 |
| Oil | 6.9 | 4.7 |
| Lysine | 0.23 | 0.66 |

# DE (Digestible energy), CP (crude protein), Ash (mineral content), NDF (neutral detergent fibre), ADF (acid detergent fibre).

# *2.1.2.* *Growth Measurements*

Both groups of foals were subject to weekly growth measurements, except for weeks 11 and 16. A number of different measurements were taken to capture any changes in body growth pattern over the 18 weeks, including BW, HG, HH, WH and BL.

Body weight measurements were taken using a Salter Bracknell PS2000 Floor Scales, Foundry Lane Smethwick, West Midlands UK. The initial 3 weeks of measurements involved repeated acclimatization to the weigh-bridge by leading the foals (all were halter trained before the trial began) over the bridge several times before taking the measurement. After this time all foals readily walked onto the scale and stood still while their weight was taken. Average Daily Gain was calculated by subtracting the previous week’s measurement from the new weight score and dividing that value by the number of days since last measured (typically 7 days except for weeks 11 and 16 when 14 days elapsed between measurements).

WH and HH were measured using a Shires Equestrian Aluminium Extending Measuring Stick, GS Equestrian, Long Road, Paignton, TQ4 7AU UK. This measurement was taken while foals were ‘stood-up square’ on a level surface of hard core just outside the field entrance, so foals were not separated from their dams at any time before weaning.

HG and BL measurements were taken using a Pet Plan Equine Measuring Tape, <https://www.petplanequine.co.uk/contact/contact.asp>. The HG in cm was taken from just behind the foal’s withers with the tape pulled taut around the foal’s body following respiratory exhalation; BL (cm) from the centre of the foal’s chest, along the side of the body to the tuber ischii.

*2.2. Trial 2 In vitro gut simulation measurements*

*2.2.1. Stomach pH measurements*

*In vitro* stomach digestion was simulated using a modification of the technique of Moore-Colyer et al [20], where incubation follows the conditions in the oesophageal, fundic and pyloric regions of the stomach. Nine glass beakers (3 replicates per treatment) were prepared with either 10 g of a) SC, b) TMFR or c) no feed control.

Using a pipette, 10ml of artificial saliva solution containing 2.86g/l sodium chloride (NaCl), 1.50g/l potassium hydrogen carbonate (KHCO3) and 2.77g/l sodium hydrogen carbonate (NaHCO3), and 200ml 0.075M HCL (adjusted to pH 6) were added to each 600ml beaker. A microprocessor pH probe (model PHB-213; Omega) was placed into the solution and each beaker was then covered with cling film and placed into a climate control cabinet on a shaker set at 95 revolutions per minute, where it was incubated for 2 hours at 37oC. The pH was recorded automatically every 30 seconds.

After 2 hours of incubation the solution was adjusted down to pH 5.4 in order to mimic the pH in the fundic region of the stomach, so 20μl of 0.075M HCl was added to each beaker. At the same time, to mimic the initial stages of protein catabolism, 0.133g of pepsin was added at a concentration of 2g/L. At 4 hours incubation in order to mimic the pyloric region of the stomach 6.13ml of 0.075M HCl and 0.266g of pepsin were added. Incubation continued for another 2 hours before the experiment was terminated.

*2.2.2. In vitro* *hind gut fermentation using the gas production technique*

The gas production technique of Theodorou et al [21] was used to measure the fermentation rate and pH profile of the two diets using the faeces from each of the 8 foals as the microbial inoculum. Twelve replicate bottles containing 1 g SC feed + 3 controls (no feed) were prepare for each of the 4 foals in SC group. Similarly 1 g TMFR was weighed into 12 replicate bottles + 3 controls (no feed) for each of the 4 foals in TMFR group, thus the experiment consisted of 120 bottles. The 12 bottles per treatment allowed 3 replicate bottles to be removed from the incubation at each time point of 4, 12, 24 and 62 hours incubation. These bottles were harvested to measure pH and lactate levels at each of these times (see method below).

Freshly voided faeces were collected into CO2 flushed pre-warmed thermos flasks from each foal. Faeces were then transported to the lab where the individual microbial inoculum from each foal was prepared using a ratio of 1:5 ie.,75g faeces to 375ml of pre-prepared Van Soest medium. The modified Van Soest culture medium was prepared [22]. Bottles containing substrate were flushed with CO2 before the addition of 70 ml of culture medium, 4 ml of freshly prepared reducing agent (2.5 g of cysteine HCl,16 ml of 1M NaOH, 2.5 g of sodium sulphate, and 380 ml of distilled water), and 10 ml of fecal inoculum. The bottles were then sealed using aluminium and rubber crimp seals and placed in an incubator at 37oC until all bottles were prepared. Once all the bottles were prepared, a syringe needle was pushed into the rubber seal of each bottle so any gas build-up was released. This was then taken as time zero. All the control bottles (those without substrate) received the same treatment

Gas accumulation measurements were taken using a manual pressure transducer technique [21]. Gas volume (ml) and pressure readings (psi) were taken at 4, 10, 16, 22, 28, 38, 46 and 62 hours post inoculation. After each reading, the bottles were shaken to ensure good contact between the microbial inoculum and food substrate. Three bottles from each foal and feed combination were removed from incubation at three time points 4, 12, 24 to determine pH and lactate levels. Gas production profiles from 3 replicate bottles per foal were allowed to continue to ferment until the end of the incubation period at 62 hours.

At the pre-designated time after the gas reading, the harvest bottles were removed from the incubator and the crimps removed. The contents of the bottle were poured into funnels containing pre-weighed filter papers. The collected liquid was used to measure pH with the probe (Thermo Fischer Scientific Orion Star A211 pH Benchtop Metre) and a 10ml sealable tube filled and placed into a freezer at -80oC for lactate testing later.

D-lactate was measured in the samples using the D-lactate Colorimetric Assay Kit (Sigma-Aldrich) on a 96 well plate. 1mM standard solution was made by 10μl of D-lactate standard solution with 990μl D-lactate assay buffer in Eppendorf tubes and mixed. These were then pipetted into the wells at volumes of 0, 2, 4, 6, 8, 10, 12 and 14μl and each topped up with D lactate assay buffer to 50μl. A second plate was run with four further series of these standards, so that an average could be taken to form the standard curve. 0.2μl of each sample was pipetted into the wells on the plate, and 2μl of both D-lactate enzyme mix and D-lactate substrate were added, as 45.8μl of D-lactate assay buffer to bring the volume of each well to 50μl. To be able to calculate background activity from NADH and NADPH, a blank was created at each time point for each horse by mixing 48μl of one of the samples with 2μl of the D-lactate substrate. Readings were taken at 450mm using a plate reader (Diagnostic Automation, DAR800).

*2.3. Data analyses*

*In vivo* data for the two groups of foals across the 17 measurement weeks were tested for normality using the Shapiro-Wilk test (Genstat 18). Data was not normally distributed, (likely due to limited numbers in each group), therefore the non-parametric Wilcoxon matched pairs test was used to test for differences between groups for live weight (LW), wither height (H), body length (BL), heart girth (HG), hip height (HH) and average daily gain (ADG) in kg, with P<0.05 as the significance level.

*In vitro* gas volume readings were corrected for pressure using linear regression [21] and summed to produce cumulative gas volumes for each bottle. The maximum likelihood programme was used to fit the France et al.[23] modelto estimate the parameters for the best fit. The fitted parameters of lag time (LT), the time to reach 50% of gas produced (t50), volume of gas produced at 50% of the total incubation time y50, and the calculated fractional rate of gas production (FRGP) were all analysed via Man Whitney U test for independent groups. Differences between the three feed treatments for the foregut data on pH was analysed by repeated measures ANOVA. Data from the hind gut gas production for pH and D-lactate were also determined using Repeated Measures ANOVA, with feed and replicate beakers as factors.

**3. Results**

*3.1. Trial 1. In vivo intake and growth measurements*

All foals readily ate both of the creep feeds offered and no major health issues were observed in any of the foals over the 18-week trial period. Across the 17-weeks of measurement the SC group consumed 3.9, 4.6, 5.3 and 5.9 kg / head per day by weeks 4, 8, 12 and 17 respectively. Foals on the TMFR ate 6.4, 7.6, 8.6 and 9.9 kg/head per day across the same time period. Table 3 below shows the final daily nutrient intake / foal they obtained from eating 6 kg of SC and 10 kg of TMFR.

**Table 3**. The final daily voluntary feed intake (VFI /f/d) of individual nutrients per foal when consuming 6kg of Stud Cubes (SC) and 10kg of total mixed fibre ration (TMFR)

|  |  |  |
| --- | --- | --- |
| Feed | TMFR | SC |
| VFI kg/f/d | 10 | 6 |
| DE (MJ) | 149 | 82.8 |
| CP (g/kg) | 155 | 96 |
| NDF (g/kg) | 313 | 133 |
| ADF (g/kg) | 185 | 45.7 |
| Oil (g) | 69 | 28.2 |
| Lysine (g) | 2.3 | 3.96 |

The ages of the foals across both groups at the end of the trial were as follows: 4 foals were 7 months, 2 were 6 months and 2 were 5 months old. The measurement period therefore covered the important fast growth phases for all the foals. In order to capture the average daily gain throughout this highly active growth phase the ‘match-pairing’ for each group was done on birth date to ensure similar potential growth profiles in each group. However due to different pedigrees this resulted in slightly different live weights for foals born on similar dates, thus the starting weight of the 4 foals in the stud cubes group was 27 kg heavier (P<0.05) compared with the 4 foals in the TMFR group as detailed in Table 4.

**Table 4.** Age and Live Weight (kg) (±SD) for 4 foals fed Stud Cubes (SC) and 4 foals fed Total Mixed Fibre Ration (TMFR) at the beginning of the trial (10th May 2017)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Foal | Foal age  (days) | SC (LW kg) | Foal | Foal age (days) | TMFR (LW kg) |
| 1 | 91 | 184 | 5 | 87 | 144 |
| 2 | 76 | 171 | 6 | 82 | 104.5 |
| 3 | 68 | 159 | 7 | 71 | 136 |
| 4 | 33 | 107.5 | 8 | 37 | 94 |
| Mean (±SD) | 67 (±24.5) | 155.4 (±33.5) |  | 69 (±22.5) | 128.6 (±23.3) |
| P-value for LW | 0.018 | | | | |

The mean live weights (LW) for SC and TMFR groups across the 18 weeks are shown in Fig 1a. At the end of the trial the mean LW for SC group was 283.5 (± 25.6) kg and TMFR group 249.6 (±21.3) thus the difference between the groups altered little during the 18-week feeding period, with SC finishing the trial 33.6 kg heavier (P<0.05) than TMFR. As is seen in Figure 1a, the growth trajectory for both groups is similar showing both diets maintained similar growth rates throughout the study.

Average daily gain (ADG) of the foals is also shown in Fig 1a and shows that SC group had an ADG of 1.1 kg/day and TMFR group of 1 kg/day thus there was no significant difference in growth rate between the two groups. As shown in Fig 1a the SD for SC was greater than for the TMFR group.

The average WH in cm at week one for SC group was 123 (±4.3) which was 6.5 cm taller (P<0.05) than the TMFR group at 117 (±4.0) Fig 1b. As noted for the ADG the difference between groups remained constant throughout the 18 week trial period with mean wither height average daily gain (WADG) of 0.7mm for the SC group resulting in them finishing at 132 (+1.9) which was 5.3 cm taller (P<0.05) than the TMFR group at 127 (+2.3) cm with an WADG of 0.8mm, Fig 1b. Thus, the SC grew by 9.1 cm and TMFR by 10cm over the 18 weeks.

The average hip height in cm at week one for SC group was 128 (±5.0) which was 5.4 cm taller (P<0.05) than the TMFR group at 123 (±4.3), Fig 1c. This difference was also maintained throughout the 18 week trial period with mean hip height for the SC group finishing at 145 (+1.8) which was 5 cm taller (P<0.05) than the TMFR group at 140 (±1.9) cm. Thus the hip height for both the SC and TMFR groups grew by 17 cm, an average daily hip height gain (ADHHG) of 1.3mm /day over the 18 week trial.

Heart girth and BL are shown in Table 5 and show that both groups grew at an even and similar rate thus both feeds supported growth equally well.

**Table 5**. Total growth and average daily growth (ADG) in heart girth (HG) and body length (BL)(cm) when 4 foals were fed a total mixed fibre ration (TMFR) and 4 foals fed stud cubes (SC) over 18 weeks

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Measurement | TMFR | Growth (cm) | ADG  cm | SC | Growth (cm) | ADG  cm |
| HG week 1 | 97 (±5.6) | 44 |  | 105 (±9.2) | 41 |  |
| HG week 18 | 141 (±5.7) | 0.34 | 146 (±5.6) | 0.32 |
| BL week 1 | 97 (±10.2) | 53 |  | 113 (±12.7) | 45 |  |
| BL week 18 | 150 (±7.4) | 0.42 | 158 (±7.0) | 0.36 |

*3.2. Trial 2. In vitro gut simulation measurements*

*3.2.1. In vitro stomach digestion*

The pH profiles for the three different feed treatments (TMFR, SC and no feed control) when incubated over 6 hours at 37oC in the *in vitro* simulated stomach conditions are shown in Fig 2. Across the 6-hour incubation period the TMFR maintained a steady but higher (P<0.05) pH with a mean of 5.84 compared with the SC with a mean of 5.33. The no feed control is a reflection of the pH of the solution and showed an immediate rise to pH 8 which was above that noted for the other two feeds. At 4.16 hours when more acid was added, an immediate drop to pH 4, which was below the SC and TMFR diets was noted and remained at this level until the end of the incubation period.

*3.2.2. In vitro* hind gut gas production

Fig. 3 shows the cumulative gas production profiles when TMFR or SC were incubated for 62 hours with faecal inoculum from each of the 8 foals, 4 fed SC and 4 fed TMFR. The curves follow a similar pattern of gas production but the SC produced nearly twice the amount of gas in half the time over the 62 hours compared with the TMFR. Both feeds also showed a lag in fermentation rate between 4 and 10 hours post inoculation.

Table 6 shows that the SC produced significantly more total gas at y50 (half-way through the incubation period) and at the end of the incubation time (A) than the TMFR. However, the lag times (L) and fractional rate of gas production (FRGP) were similar between the two feeds showing that the total quantity of fermentable constituents was greater in the SC than TMFR but that they both were similar in their speed of degradation.

**Table 6.** Parameters calculated using the France *et al.* (1993) model for fractional rate of gas production (FRGP), total gas pool (A), lag time (L), time to produce 50% gas (t50) and amount of gas produced at half incubation time (y50) when stud cubes (SC) or total mixed fibre ration (TMFR) were incubated for 63 hours with foal fecal inoculum from 4 ponies on the corresponding diets

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | SC | TMFR | P-value |
| FRGP (ml/hr) | 0.0373 | 0.0301 | 0.343 |
| A (ml) | 158.8 | 91.22 | 0.029 |
| Lag (hrs) | 0.12 | 0.21 | 1.00 |
| t50 (hrs) | 11.64 | 24.57 | 0.029 |
| y50 (ml) | 79.39 | 45.61 | 0.029 |

Fig.4 shows the pH changes in the bottles over 58 hours incubation, with the TMFR increasing (P<0.05) in pH from its lowest of 6.75 at 12 hours up to 6.89 at 62 hours. This was significantly (P<0.019) higher than the SC feed which dropped to 6.61 at 24 hours and raised slightly to 6.71 at 62 hours.

Lactate levels are depicted in Fig 5 and show the TMFR levels to be constant throughout the 62-hour incubation, starting at 0.28 and finishing at 0.26 mM/l SC on the other hand produced 0.8 mM/l after 12-hours of incubation and dropped down to 0.18 mM/l at the end of the incubation.

**4. Discussion**

*4.1 Trial 1. In vivo intake and growth measurements*

The objectives of the *in vivo* part of this trial were to determine if foals would readily eat an all-fibre creep feed (TMFR) and if such a feed could support the fast growth rates traditionally sought from Thoroughbred (TB) foals, destined for a career in racing. Although this was the first controlled trial of the TMFR, the design of this experiment was based on the findings of the previous season’s testing at Bucklands Stud when 12 TB foals were offered the TMFR as their sole creep feed and maintained good growth rates. Therefore, the practicalities of feeding the TMFR, such as feeding system (see Plate 1) and amount to feed were all based on previous *in vivo* data. The results reported here showed that foals readily accepted both feeds and the level of intake supported the high ADGs of 1 to 1.1 kg/day. This high growth rate can be partially explained by the age range of the foals in this experiment who started the trial at 1-3 months old and completed it between 5 and 7 months old, thus all the foals were in their rapid growth phase. The foals in this experiment achieved higher growth rates than were observed in a group of 885 TB foals in Kentucky [24] who had an ADG of 0.89 and 0.82 at 4 and 6 months old respectively. In both the present study, and that of Ringler and Lawrence [24] the foals grew faster than the recommended level of 0.94kg/d for 2 month old foals and 0.64 kg/d for 7 month old foals [25]. Jeffcott [26] stated that there was a lower incidence of DOD in foals when they were fed according to the NRC 1989 recommendations which were set at 0.85 kg/d for rapid growth [27]. These recommendations were set within ‘safe-limits’ to avoid developmental orthopaedic disease (DOD). Despite the notably faster growth rates recorded in the present study and the fluctuations in ADG between some of the weeks (as shown in Fig 1a) the foals demonstrated the signs of good health, eg. shiny coats, bright eyes, active characters, had ‘visibly clean’ joints, and were sound.

Both feeds were specifically designed to be fed to young growing TB. The Sharp Nutrition Stud Cubes (SC) were the preferred concentrate used at Buckland’s Stud as it was specifically formulated to have high DE and CP and low fibre contents so it provided readily digestible nutrients to support rapid growth rates. The stud had achieved good results in previous seasons using this feed so it was decided to use this as the concentrate feed for group A. The amount of feed given to each group of foals was controlled by the stud owner, and were set to achieve the growth rates she wished for within a given time period. Thus, by the end of the trial, the respective foal groups were being fed 10 kg /foal / day of TMFR, which provided 149 MJ DE/day, and 6kg of SC diet which provided 83 MJ DE/day. Both these rations provided more than the 69 MJ DE fed to 7 month old warmblood foals by Mack *et al.,* [28] and were also above the 78 MJ/day recommended by NRC [25] and the 74MJ DE per day allowance recommended by the German Society of Nutrition Physiology in 1994 for foals from 7 to 12 months of age. This resulted in the TB foals in this study consuming more energy on both diets despite being of similar age and size to the warmblood foals fed by Mack et al [28]. However, comparing energy intakes across diets is potentially problematic as the proportion of cereals to fibre and the fibre type, maturity, particle size and processing, will influence the heat increment and thus the conversion of DE to net energy (NE) which is available for maintenance and growth [29, 30]. Thus the TMFR, albeit a highly digestible fibre feed, would yield proportionally less NE/ kg than the cereal based SC diet. This in combination with the higher maintenance requirements, [31] and higher growth potentials seen in TB foals compared with other breeds contributed to their higher growth rates without them becoming over weight or showing any visible signs of skeletal growth abnormalities.

Over-feeding energy and protein to young growing animals can initiate DOD, especially OCD, particularly when feeding high amounts of cereal-based concentrates. Savage et al [32] found that foals fed 128 % of NRC 1989 recommendations, by feeding excessive carbohydrate and oil had widespread OCD lesions. The ADG of the warmblood foals in the Mack et al [28] trial from 190 days old to 235 days old (45 day period) was approximately 0.733 kg /day and was deemed to put these foals ‘at risk of potential growth disorders’ so in month 8 the diets were reduced to 58.5 MJ DE /day. Both Warmbloods [33] and TB are susceptible to DOD [11, 34] and there is a significant genetic component to this condition [35]. Perhaps the foals in Mack et al [28] study were a susceptible group and with the growth rate slowing down at 7 and 8 months old the level of energy intake might indeed have put them at risk. However, the group of TB used in our study were younger and in the most rapid phase of growth and this combined with the strong possibility that they were not a genetically predisposed DOD group, resulted in them being able to tolerate a much higher level of feed intake for a total of 126 days without any visible problems.

A limitation to this study was that individual feed intakes were not measured as animals were group fed according to the practices on the stud farm. However the data was obtained in real field conditions and is therefore of practical use and relevance to other TB breeders. The amount of TMFR eaten per foal per day by the end of the trial (10kg) was well within the 2.5% of BW in DM /day recommendations [25]. While this may seem a large amount of feed, none of the foals showed potbellies or excessive build-up of body fat. In fact the growth achieved over the 126 day feeding period was greater at the withers, heart girth and length for the TMFR group than for the SC group. Indeed the growth for heart girth and wither height for both TMFR and SC were notably greater than those reported previously [36] in 128 TB foals in Florida, who reported ADG for foals from 112 days to 181 days (69 day period) for heart girth of 0.1cm, wither height of 0.086 cm, BW 0.55 kg and hip height 0.08 cm, compared with the 0.34, 0.08, 1 kg and 0.13cm recorded respectively for the TMFR in this study. Lepeule [7] reported an increased odds ratio for DOD and OCD in Warmblood foals who grew quickly and attained a greater wither height than TB foals on a similar trajectory, suggesting that the fast-growing Warmbloods were more susceptible to DOD than the TB.

Both feeds supplied significantly more CP (TMFR = 1550 g/d and SC= 960g/d) than the recommended 688g/d set by the NRC 2007 [25] for 7 month old foals. Therefore, both feeds were supplying plenty CP to sustain high growth rates. To-date no relationship has been found between feeding excessive protein and DOD. In fact the data reported by Savage et al [32] showed that when foals were fed 126 per cent of the NRC [25] requirements for protein, no DOD lesions were found. In contrast, 50% of the foals fed 129 per cent of the NRC requirements for DE consistently showed OCD lesions. Elevated insulin is implicated in the pathogenesis of OCD [37], supported by diets that have a high glycaemic index e.g. high starch and sugar content (typical of the more traditional stud and young stock rations) are much more likely to develop OCD.

These results dismiss the industry held belief that young growing TB must receive cereals to achieve maximum growth rates and in fact clearly indicate that a high quality specifically balanced high fibre diet can support growth in young TB achieving similar BW gains compared with a high cereal diet. The foals in this trial did not show any visible signs of DOD when fed at this level. However, the limited numbers of foals in each group mean that caution should be exercised on feeding levels, as this group of foals may not be fully representative of the TB population and other foals with inherent susceptibility to DOD may not tolerate this level of feeding.

*4.2.**In vitro gut simulation measurements*

The results from both the *in vitro* stomach and hind gut simulation trials showed that the TMFR maintained a more constant and higher pH and produced lower lactate levels in both simulated regions compared with the SC feed. This suggests that the TMFR has potential to help reduce the incidence of acid precipitated gastric ulceration and promote a hind gut environment conducive to healthy microbiome maintenance.

The methodology of the stomach simulation was designed to mimic food passing through the pH gradients that occur in the different regions of the stomach [38], thus small pH drops were seen when additional HCl was added to the beakers. However the pH drop quickly rose again and pH remained constant for both feeds, although lower for SC than TMFR. Some hydrolysis of both feeds was obviously occurring as the pH for both diets dropped compared with the no-feed control. Nutrient disappearance was not measured in this experiment which is a limitation to the interpretation of what was occurring as a result of addition of acid and pepsin. Nevertheless, the TMFR diet maintained a higher (P<0.05) pH during the entire 6-hour incubation compared with the SC showing that either less hydrolysis or less protein digestion was occurring from the TMFR compared with the SC diet. The pH difference may also be the result of the release of different ions from TMFR compared with the SC feed which resulted in a better buffering and a higher pH. After the second addition of acid at 4 hours the pH of the non-feed control dropped to pH 4 which is required *in vivo* to cause the activation of procarboxy peptidase to pepsin so protein breakdown can be initiated [39]. TMFR and SC feeds were clearly buffering this pH drop as they quickly raised the pH to 5 in SC feed and up to 5.5 in the TMFR. While this might be seen as a negative consequence of stomach buffering, breakdown of protein in this *in vitro* model was not constrained by pH level as active pepsin was added so protein breakdown could occur. *In vivo* the desired pH drop would be facilitated by the action of lactic acid bacteria which initiate fermentation of feed and produce lactic acid, further dropping the pH of the stomach environment. As the objective of this trial was primarily to compare the buffering capacity of the TMFR with SC the addition of lactic acid bacteria as per the procedure of Moore-Colyer and Jiang [40] was not performed.

The total gas productions from both feeds reflects their potential degradability and are similar to previous work comparing fibre with cereal feeds [41, 42], where the more degradable cereal feeds produce significantly more gas than the fibre feeds. All other parameters recorded for the TMFR feed are in broad agreement with earlier work although lag time in this trial was notably shorter than previously recorded for a high fibre feed [40]. This could be attributed to the fact that the faecal inoculates used for both diets were from foals specifically adapted to these diets and thus microbial profile would have been compatible with the substrate in the bottles.

The pH for both TMFR and SC feeds was notably higher and the lactates lower than the values previously noted [40] for high fibre and high cereal feeds. Over the 62 hour incubation the pH and lactate concentrations of the TMFR remained relatively constant but the SC feed showed a significant drop from 4 to 24 hours for pH and from 12 to 62 hours for lactate. However the pH for both feeds is well above the level noted to be the threshold for acidosis in the equid hind gut [43].

The drop in pH and higher lactates noted in the early stages (4 - 24 hours) post inoculation reflects the degradation of the readily degradable components in both feeds. The lactate levels produced by the SC feed were more than double those produced by the TMFR and can be explained by the readily degradable starch content in the SC feed. The TMFR feed raised the pH at approx. 13 hours and the lactate levels remained constant throughout the entire incubation demonstrating the absence of starch in this feed. Thus overall the composition of the TMFR feed provided readily degradable substrate but the components resulted in slower degradation and limited production of potentially damaging lactate.

**Conclusions**

Results from this study demonstrate that high growth rates can be achieved by TB foals when fed a high-fibre creep feed (TMFR) and these growth rates are comparable to those achieved when a conventional cereal-based creep feed is fed. *In vitro* results suggest that the TMFR will maintain a healthy gut environment by raising pH and lowering lactate production. The result here dispel the industry-held belief that feeding high levels of fibre to foals will produce pot-bellies and not sustain desired growth rates. In fact the range of body growth measurements taken here indicated that the TMFR supported growth in rapidly growing TB foals in a similar way to that seen in foals fed conventional cereal-based concentrate creep feed.

**Acknowledgements**

The authors would like to acknowledge Roisin Close and the staff of Bucklands Farm and Stud for supplying the animals for this trial, the daily management of the foals and assisting with weekly measurements. Thanks also to Eclipse feeds Innishannon, Co Cork Ireland for supplying the TMFR for the trial.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Eclipse feeds Innishannon, Co Cork, Ireland supplied the TMFR for the trial.

**References**

[1] Brown-Douglas CG, Pagan JD. Body weight, wither height and growth rates in Thoroughbreds raised in America, England, Australia, New Zealand and India, In: Proceedings of the 15th Annual KER Conference, Kentucky Equine Research, Lexington, 2006: pp.15-22.

[2] Pagan JD, Brown-Douglas CG, Caddel S. Body Weight and Condition of Kentucky Thoroughbred Mares and their Foals as Influenced by Month of Foaling, Season, and Gender. Publication-European Association for Animal Production 2006; 120: 245.

[3] Luszczynski J, M Pieszka. Growth Rate of Thoroughbred Horses During First Six Months of Life, Scientific Information Database 2011; pp.131-134.

[4] Dicks L, Botha M, Dicks E, Botes M. The equine gastro-intestinal tract: An overview of the microbiota, disease and treatment, Livestock Science; 2014: 160, pp.69- 81.

[5] Verveurt I, Voigt K, Hollands T, Cuddeford D, Coenen M. Effect of Feeding Increasing Quantities of Starch on Glycemic and Insulinemic Responses in Healthy Horses. Equine Veterinary Journal, 2009: 182 pp.67-72.

[6] Coenen M. Beobachtungen zum Vorkommen futterungsbedingter Magenulcera beim Pferd. SchweizerArchiv fur Tierheilkunde. 1990: 132, (3), 121-126

[7] Lepeule J, Bareille N, Robert C, Ezanno P, Valette JP, Jacquet S, Blanchard G, Denoix JM, Seegers H. Association of growth, feeding practices and exercise conditions with the prevalence of developmental orthopedic disease in limbs of French foals at weaning. Prev. Vet. Med. 2009; 89: 167-177

[8] Elfenbein JR, Sanchez LC, Prevalence of gastric and duodenal ulceration in 691 non surviving foals(1995-2006). Equine Veterinary Journal 2012; Suppl 41: 76-79

[9] Murray MJ, Gastric ulcers in horses: Endoscopic appearance of gastric lesions in foals: 94 cases (1987-1988) J. Am. Vet. Med. Assoc. 1989; 195: 1135-1141

[10] Secombe CJ, Lester G.D. The Role of Diet in the Prevention and Management of Several Equine Diseases. Animal Feed Science and Technology 2012; 173: 86-101.

[11] Pagan J D, Geor R J, Caddel S E, Pryor P B and Hoekstra K E. The relationship between glycemic response and the incidence of OCD in thoroughbred weanlings: a field study. 47th Annual American Association of Equine Practitioners Convention, 2001; San Diego, California.

[12] van Weeren PR. (2006) Etiology, Diagnosis, and Treatment of OC(D). Clinical Techniques in Equine Practice 2006: 5(4): 248-258.

[13] Jeffcott LB, Henson FM. Studies on Growth Cartilage in the Horse and Their Application to Aetiopathologenesis of Dyschondroplasia (Osteochondrosis), Veterinary Journal 1998; 156:177-192.

[14] Robert C, Valette JP, Jacquet S, Denoix JM. Influence of juvenile oesteochondral conditions on racing performance in Thoroughbreds born in Normandy. The Vet Journal 2013; 197: 83-89.

[15] Kane AJ, Park RD, McIlwraith CW, Rantane N.W, Morehead JP, Bramlage LR. Radiographic changes in Thoroughbred yearlings. Part 1. Prevalence at the time of the yearling sales. Equine Vet. J. 2003; 35: 354–365

[16] Kabe M.G, de Souza AD, de Moro Sousa RL, da Silva Bueno IC, Mota TP, Crandell K, Vervuert I, Correa GF, Brandi RA. Soybean Hulls in Equine Feed Concentrates: Apparent Nutrient Digestibility, Physicochemical and Microbial Characteristics of Equine Feces. Journal of Equine Veterinary Science 2016; 36:77-82.

[17] Newbold J, Dougal K. What do changes in the microflora with diet potentially mean for the animal In. ESCVN Congress Cirencester 2017 Proceedings: 22-25.

[18] Saastamoinen M, Särkijärvi S. Digestibility of a forage-based diet in weanling horses during development and maturation, Livestock Science.2017; 215: 49-53

[19] [Camacho-Luna P](https://www.ncbi.nlm.nih.gov/pubmed/?term=Camacho-Luna%20P%5BAuthor%5D&cauthor=true&cauthor_uid=29534810), [Buchanan B](https://www.ncbi.nlm.nih.gov/pubmed/?term=Buchanan%20B%5BAuthor%5D&cauthor=true&cauthor_uid=29534810), [Andrews FM](https://www.ncbi.nlm.nih.gov/pubmed/?term=Andrews%20FM%5BAuthor%5D&cauthor=true&cauthor_uid=29534810). Advances in Diagnostics and Treatments in Horses and Foals with Gastric and Duodenal Ulcers. [Vet Clin North Am Equine Pract.](https://www.ncbi.nlm.nih.gov/pubmed/29534810) 2018; 34(1):97-111.

[20] Moore-Colyer MJS, O’Gorman D, Wakefield K. An *in vitro* investigation into the effects of a marine-derived multi-mineral supplement in simulated equine stomach and hindgut environments. Journal of Equine Veterinary Science. 2014; 34(3): 391-397.

[21] Theodorou M K, Williams BA, Dhanoa, MS, Mc Allan, AB. France J. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds’, Animal Feed Science and Technology 1994; 48: 185–197.

[22] Theodorou MK, Brooks AE. Evaluation of a new laboratory procedure for estimating the fermentation kinetics of tropical foods. Contractor report EMC X0162. 1990; Aberystwyth, Wales UK: Institute of Grassland and Environmental Research

[23] [France](https://www.sciencedirect.com/science/article/abs/pii/S0022519383711094#!) J, [Dhanoa](https://www.sciencedirect.com/science/article/abs/pii/S0022519383711094#!) MS, [Theodorou](https://www.sciencedirect.com/science/article/abs/pii/S0022519383711094#!) MK, [Lister](https://www.sciencedirect.com/science/article/abs/pii/S0022519383711094#!) SL, [Davies](https://www.sciencedirect.com/science/article/abs/pii/S0022519383711094#!) DR, [Isac](https://www.sciencedirect.com/science/article/abs/pii/S0022519383711094#!) D. A Model to Interpret Gas Accumulation Profiles Associated with In Vitro Degradation of Ruminant Feeds. Journal of Theoretical Biology 1993; 163: 99-111

[24] Ringler MS, and Lawrence LM. Comparison of Thoroughbred Growth Data to Body Weights Predicted by the NRC. Journal of Equine Veterinary Science 2008; 28: 97-101

[25] National Research Council RC (2007). Nutrient requirements of Horses 6th edition. National Academies Press. Washington DC USA 2007.

[26] Jeffcott L.B. Osteochondrosis – an international problem for the horse industry. J. Equine Vet. Sci. 1996; 16: 32–37.

[27] National Research Council. Nutrient requirements of Horses 5th edition. National Academies Press. Washington DC USA 1989.

[28] Mack JK, Remler HP, Senckenberg E, Keinzle E. No effect of moderate or high concentrate allowance on growth parameters in weanling warmblood foals fed late cut hayalge as forage. Animal Physiology and Animal Nutrition. 2014; 98: 886-893.

[29] Frape D. Equine Nutrition and Feeding. 4th edition. 2010; Wiley Blackwell Publishing Ltd. 9600Garsington road Oxford, UK

[30] [Vermorel](https://www.sciencedirect.com/science/article/pii/S0301622696014029#!)M**,** [Martin-Rosset](https://www.sciencedirect.com/science/article/pii/S0301622696014029#!)W,[Vernet](https://www.sciencedirect.com/science/article/pii/S0301622696014029#!)J. Energy utilization of twelve forages or mixed diets for maintenance by sport horses [Livestock Production Science](https://www.sciencedirect.com/science/journal/03016226) 1997; 47: 157-167

[31] Keinzle E, Zeyner A. The development of a metabolisable energy system for horses. Journal of Animal Nutrition and Animal Physiology 2010; 98: 231-240.

[32] Savage CJ, McCarthy RN, Jeffcott LB. (1993) Effects of Dietary Phosphorus and Calcium on Induction of Dyschondroplasia in Foals. Equine Veterinary Journal Supplement 1993; 16: 80-83.

[33] Hoppe F. Radiological investigations of osteochondrosis dissecans in Standardbred Trotters and Swedish Warmblood horses. Equine Veterinary Journal 1984; 16(5): 425-429.

[34] Pagan J D. The incidence of developmental orthopaedic disease (DOD) on a Kentucky thoroughbred farm, Advances in Equine Nutrition, Nottingham University Press 1998; 469-475.

[35] Castle K. Investigating the Genetic and Genomic Basis of Osteochondrosis in Thoroughbred Horses from Australia and New Zealand, PhD Thesis 2012; University of Sydney, Sydney, New South Wales.

[36] Kavazis AN, Ott EA. Growth Rates in Thoroughbred Horses Raised in Florida. Journal of Equine Veterinary Science 2003; 23:(8) 353-357

[37] Henson FM, C. Davenport C, L. Butler L, Moran I, Shingleton WD, Jeffcott LB, Schofiled PN. Effects of insulin and insulin-like growth factors I and II on the growth of equine fetal and neonatalchondrocytes. Equine Vet. J. 1997; 29: 441-447.

[38] Jassim A, Andrews FM.The bacterial community of the horse gastrointestinal tract and its relation to fermentative acidosis, laminitis, and stomach ulcers. Vet Clin North Am Equine Pract. 2009; 25(2):199-215.

[39] Geor R, Coenen M, Harris P. Equine Applied and Clinical Nutrition: Health, Welfare and Performance. 2013; ISBN 9780702054181 Online access

[40] Moore-Colyer MJS,and Jiang C, Preliminary development of a dynamic gastric *in vitro* model for horses *Proceedings of the 8th* European Workshop on Equine Nutrition, Dijon June 2016.

[41] Murray JMD, Moore-Colyer MJS, Dunnett C, Longland AC. The effect of feeding a low or high starch diet on equine faecal parameters. Livestock Science 2014; 159: 67-70

[42] Bice RKT, Moore-Colyer MJS. The effect of particle size on volatile fatty acid profiles obtained from an alfalfa and unmolassed sugar beet pulp diet following in vitro incubation with equine faeces. *Proceedings of the Emerging Equine Science Conference, Cirencester, UK Sept 2003.*

[43] Radicke S, Kienzle E, Meyer H. Preileal apparent digestibility of oats and corn starch and consequences for caecal metabolism. In: Proceedings of 12th Equine Nutrition and Physiology Society 1991; 43-48.

[44] Moore-Colyer MJS, Morrow HJ, Longland AC. Mathematical modelling of digesta passage rate, mean retention time and *in vivo* apparent digestibility of two different lengths of hay and big bale grass silage in ponies. British Journal of Nutrition.2003; 90: 109-118.



Foal 4

Foal 3

Foal 2

Foal 1

11 12 13 14 15

9 10

6 7 8

WEEK

4 5

3

2

1

270

250

230

210

190

170

150

130

110

90