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Original Research

An In Vitro Investigation into the Effects of a Marine-Derived, Multimineral Supplement in Simulated Equine Stomach and Hindgut Environments

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ABSTRACT

Management of the performance horse often incorporates meal feeding of highly digestible starches and reduced access to high-fiber forage. Such regimens are associated with equine gastric ulceration syndrome (EGUS) and can alter hindgut homeostasis. In-feed buffering of gastric contents and promotion of energy derivation from high-fiber forage in the hindgut are therefore desirable properties of a nutritional supplement. A marine-derived, multimineral supplement with known buffering properties containing calcium, magnesium, and short-chain fructo-oligosaccharides (scFOS) was tested under in vitro simulations of equine stomach and hindgut conditions. Six fiber:concentrate diets were incubated for 4 hours with or without the supplement at 37°C in pepsin HCl solution adjusted to pH 4.1 and 2.6. pH was measured at 1, 2, and 4 hours postincubation. Highest overall pH values were observed with the high cereal feeds; however, the supplement significantly increased ($P < .001$) the pH across all feeds by 0.17 and 0.19 for feeds incubated at pH 4.1 and 2.6, respectively. A gas production technique was used to measure the fermentation of four fiber:concentrate diets with and without additional supplement, using equine feces as the microbial inoculum. Addition of the supplement decreased ($P < .05$) the lag time and increased the initial fermentation rate, although as the incubation continued, this effect was reduced. These results demonstrate that the supplement had a significant buffering action for 4-6 hours under simulated in vitro stomach digestion conditions and also stimulated in vitro hindgut fermentation activities.

1. Introduction

Equine gastric ulceration syndrome (EGUS) is common in performance horses, with prevalence rates of approximately 90% described in both racehorses in training [1,2] and in advanced-level competition endurance horses [3]. Lower (58%) prevalence of gastric ulceration has been reported in show horses [4], and EGUS has even been reported in (53%) nonperformance horses that are not involved in intense work [5]. Clinical signs of EGUS vary from horse to horse but may include colic and poor body condition (ill-thrift) [6]. Intense training regimens and the associated high energy requirements of performance horses dictate that modern management practices commonly combine stabling, limited grazing, and forage intake, along with meal feeding of high-concentrate, low-fiber diets. Such management practices deprive the horse of the buffering effects of a protective fiber mat and a nearly continuous flow of bicarbonate-rich saliva associated with trickle-feeding. Controlled feed deprivation with intermittent feeding, a protocol designed to expose the susceptible

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squamous gastric epithelium to periods of lowered gastric pH, is an established model for ulcer generation in an experimental setting [7].

Although an effective and approved pharmacologic therapy (omeprazole paste) exists for the treatment of EGUS [8], the feeding of supplements that purport to have a short-term gastric buffering action is gaining popularity as a preventive measure against EGUS. Whereas management changes to incorporate diets higher in fiber are always recommended in cases of EGUS, cereal feeding is often continued because it supplies fast-release energy and reduces gut weight associated with water-holding fibers [9].

It is a widely accepted recommendation that horses should be fed a minimum of 1% of body weight per day of fiber, in the form of forage or dry pasture, in order to maintain optimal intestinal function [10]. Long fiber (hay or haylage) can be a valuable source of energy, provided hindgut conditions promote cell wall degradation, thus enabling fiber to make a valuable contribution to the nutrient content of the diet. It is reported that the end products of fiber fermentation, volatile fatty acids (VFAs), supply 30% of the energy used by a horse's limb during rest [11] and that approximately 60% of total glucose produced by the horse is synthesized from the colon-derived VFA propionate [12]. It follows, therefore, that improving fiber fermentation in the hindgut, allowing VFA derivation from fiber rather than rapidly digestible starch, should provide considerable benefit to the energy balance of the horse. High-fiber diets generally avoid the problems associated with feeding high doses of rapidly digestible starch, which can cause changes in hindgut bacterial populations and clinical disease including laminitis and diarrhea [13].

In-feed buffering and digestive aids are appealing to owners as a means of disease prevention. A multimineral feed supplement derived from the *Lithothamnion* species of red marine algae is evaluated in this study. The supplement is already in use as a rumen buffer [14] and has previously been shown to reduce diet-induced inflammation of the gastrointestinal tract [15] and colitis in mice [16]. The supplement contains natural buffers such as calcium and magnesium as well as a short-chain fructo-oligosaccharide (scFOS) pre-biotic. Fructo-oligosaccharides have previously been shown to have beneficial effects on in vivo hindgut fermentation in horses [17]. This marine-derived supplement has already been shown to affect markers of bone turnover in yearling Arabians [18], suggesting that the product has systemic bioavailability extending beyond the intraluminal effects being tested in these experiments.

Initial testing, as outlined in this paper, was performed in an in vitro setting in order to acquire preliminary data that could form a basis for future live horse trials.

It is hypothesized that the marine-derived, multimineral will be an effective buffer under in vitro foregut conditions and will affect fermentation in a manner that promotes fiber digestibility in the hindgut. The objectives of these experiments were, first, to determine the in vitro buffering activity of a marine-derived multimineral supplement under conditions simulating gastric digestion in the horse and, second, to measure the fermentation activity of a range of fiber and concentrate diets in the presence and absence of the supplement, to determine whether an effect on in vitro fermentation kinetics can be detected.

Table 1
Nutrient contents of the four feeds and marine supplement[a]

Content	HFF	HCF	Alfa-A	SBP	SUP
DE (MJ/kg) Estimated	13.1	13.6	10	10.5	
CP	130	130	173	96	
OM	914	938	824	818	
Starch[b]	102	300	7	52	
NDF	343	157	480	417	
Na	2.7	2.7	2	0.6	
K	12.6	9.4	38	5.6	
Ca	13.1	9.6	17	7.6	22%
P	4.1	5.3	2.8	0.8	
Mg	2.8	2.6	3.1	2.2	5.5%
scFOS					4.75%
Ash					67.75%

CP, crude protein; DE, digestible energy; DM, dry matter; DP, digestible protein; NDF, neutral detergent fibre; OM, organic matter; scFOS, short-chain fructo-oligosaccharides.

[a] Comparison of nutrient content (g/kg DM, unless otherwise stated) of the four feeds, high-fiber feed (HFF), high-cereal feed (HCF), Alfa-A, and sugar beet pulp (SBP), and the marine supplement (SUP). All values were obtained from the analyses lists on the back of the bags of feed.

[b] Values were supplied by the feed manufacturer.

2. Materials and Methods

The marine-derived, multimineral supplement containing scFOS, and sold under the brand name EquMin Plus was provided by Marigot Ltd, Cork, Ireland. It contains a minimum of 22% calcium, 5.5% magnesium, and 4.75% scFOS. The high-fiber feed (Releve; Saracen Horse Feeds, UK) was composed of highly digestible fiber, soya hulls, oils, yeast, and vitamins and minerals; and the high-cereal feed (Race Mix; Saracen Horse Feeds, UK) was composed of a blend of cereal grains, sugar beet pulp, soya oil, and vitamins and minerals. Alfa-A was purchased from Dengie Horse Feeds, UK, and the sugar beet pulp was purchased from Trident Feeds, Ireland. The following six diets (compiled from the above-described four feeds) were assessed in experiment 1: 100% high-fiber feed (HFF); 70:30 fiber:cereal mixture (High F:C); 30:70 fiber:cereal mixture (Low F:C); 100% high-cereal (concentrate) feed (HCF); 100% Alfa-A (AA); and 100% sugar beet pulp (SBP). HFF, High F:C, Low F:C, and HCF were isocaloric and isonitrogenous, whereas the AA and SBP diets contained lower energy levels and higher and lower protein levels, respectively. Because of equipment restrictions (replicate number of bottles) the following four diets were assessed in experiment 2: HFF, High F:C, Low F:C, and HCF. Feed composition can be seen in Table 1.

2.1. Experiment 1

In vitro stomach digestion was simulated using a modification of the technique of Furuya et al [19], where a pepsin HCl solution, adjusted to pH 2.6 and 4.1, is used to reflect the variable conditions in the glandular region of the equine stomach. Each of the diets (5 g) was measured into glass beakers in duplicate, in the presence or absence of 0.05 g of supplement, thus the supplement composed 1% of

the diet. Then, 100 mL of pepsin HCl was added to each beaker and adjusted to either pH 4.1 or pH 2.6 and incubated for 4 hours at 37°C. The pH was recorded using a microprocessor pH meter (model PHB-213; Omega) at 1, 2, and 4 hours. Results were subjected to repeated measures analysis of variance. Differences between reading times, feeds, and the presence or absence of supplement was determined using a least significant difference (LSD) test where LSD = t (error degrees of freedom) × s.e.d (standard error of difference).

2.2. Experiment 2

Hindgut fermentation was simulated by using a previously published in vitro gas production technique [20] with equine feces as the microbial inoculum. Four replicate bottles of each of the four diets were prepared. Two bottles from each diet had added supplement while the other 2 had feed only, totaling 16 treatment bottles. In addition, eight control bottles were prepared. These bottles had no feed or supplement added but contained medium and fecal inoculum only. Control bottles were used to determine gas production from the fecal inoculum alone. Bottles were fermented with equine fecal inoculum at 39°C in an incubator for 68 hours.

2.2.1. Feed Preparation for Gas Production

In order to accurately reflect the in vivo digestive process and allow better inference of how the supplement might behave in the digestive tract after passage through the foregut and having undergone stomach and small intestine enzymatic digestion, 5 g of the supplement was subjected to the following in vitro foregut digestion procedure [19]. Twenty milliliters of pepsin HCl solution (2 g of pepsin/L of 0.075 M HCl) was added per gram of food dry matter (DM). The sample mixture was incubated at 37°C for 2 hours before the pH was adjusted to 7, using 2 M NaOH. The mixture was then filtered through a Buchner funnel fitted with porosity 3 filter paper, and the filtrate was discarded. One liter of NaAc buffer was added to the neutralized sample and left to equilibrate for 20 minutes. Pancrex V (Pains and Bryne Ltd, West Byfleet, Surry, UK) was added to the buffered food at the rate of 1 tablet/5g of sample DM. The mixture was then incubated at 37°C for 2 hours and stirred at 20-minute intervals. After 2 hours, the mixture was again filtered, washed with 3 volumes of water and 1 of acetone, and allowed to dry over night at 60°C. Then, 1 g of each diet was placed into prelabeled 125-mL serum bottles, and 0.02 g (to ensure weighing accuracy) of supplement was then added. This equates to 2% and doubles the manufacturer's recommended feeding rate.

2.2.2. Preparation of Culture Medium, Gas Bottles, and Microbial Medium

The modified Van Soest culture medium was prepared as previously described by Theodorou and Brooks [21] and stored in a refrigerator at 4°C until required. Bottles were flushed for approximately 4 seconds with CO_2 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 1 M NaOH, 2.5 g of sodium sulphate, and 380 mL of distilled water), and 20 mL of fecal inoculum. The bottles were then sealed using rubber stoppers and incubated at 39°C. Freshly voided horse feces from a horse consuming a mixed diet of forage and concentrates (ad libitum hay plus two meals per day of 0.5 kg of AA, 0.12 kg of SBP, and 0.5 kg of naked oats) were collected and immediately prepared under continuous CO_2. Feces (100 g) samples were mixed with 1 liter of modified Van Soest medium before being filtered through muslin. Each bottle then had 20 mL of inoculum added to it. Bottles were then adjusted to ambient pressure using the pressure transducer, and the time was noted. The eight control bottles received the same treatment but did not contain substrate.

2.2.3. Gas Accumulation Measurements and Dry Matter Loss

Readings were taken using a manual pressure transducer technique [20]. Gas volume and pressure readings (psi) were taken at 6, 12, 18, 23, 28, 34, 44, 56, and 68 hours after inoculation. After each reading, the bottles were shaken to ensure good contact between the microbial inoculum and food substrate. After the last gas reading, the contents of each bottle were filtered and washed with 20 mL of distilled water and dried at 60°C until constant weights were reached. The weight of the residue was noted and the dry matter loss calculated.

2.2.4. Data Analysis

Gas volume readings were corrected for pressure by using linear regression [20] and summed to produce cumulative gas volumes for each bottle. The maximum likelihood programme was used to estimates the parameters for the model of best fit. The fitted [22] parameters of lag time (L_T), the time to reach 50% of gas produced (t_{50}), time to reach 95% of the gas produced (t_{95}), % of DM loss, extent of DM loss (Ext D), and the calculated fractional rate of gas production (FRGP) were all analyzed via analysis of variance so that the effects of diet and the addition of the supplement on fermentation parameters could be measured. Differences between feeds and treatments (without [−] supplement and with [+] supplement) were compared using the least significant difference test.

3. Results

3.1. Experiment 1

The results shown in Table 2 indicate that the pH across all feeds rose by 0.06 ($P < .001$) after the first hour of incubation in a pepsin HCl solution adjusted to pH 4.1 but thereafter remained the same.

Feeds showed significantly ($P < .001$) different pH values across the 4-hour incubation with Alfa A having the lowest pH at 6.0, which was similar to SBP at pH 6.1. SBP, the high-fiber feed (HFF), and the High F:C mixture were all similar, whereas the high-cereal feed (HCF) had a significantly higher pH than all the other feeds except the Low F:C mixture at 6.4.

Across all time periods and feeds under moderate (pH 4.1) acidic conditions, the addition of the marine supplement raised the pH by 0.2 ($P < .001$).

Table 2
pH of six different diets incubated with pepsin at pH 4.1[a]

Time	1 hour	2 hours	4 hours	s.e.d	Sig				
pH	6.16[a]	6.22[b]	6.22[b]	0.006	***				
Feed	HFF	High F:C	Low F:C	HCF	Alfa A	SBP	s.e.d	Sig	
pH	6.18[bc]	6.22[bc]	6.28[cd]	6.39[d]	5.99[a]	6.11[ab]	0.049	***	
Treatment		Feed alone		Feed + supplement		s.e.d	Sig		
		6.11[a]		6.28[b]		0.028	***		

HCF, high-cereal feed; HFF, high-fiber feed; High F:C, 70:30 fiber:cereal mixture; Low F:C, 30:70 fiber:cereal mixture; SBP, Alfa A and sugar beet pulp; s.e.d, standard error of difference; Sig, significance level.
[abcd]Values in the same row not sharing common superscripts differ significantly ($P < .001$).
[a] Table compares pH of six different fiber and concentrate diets incubated in vitro with a pepsin HCl solution of pH 4.1 for 4 hours with (+) or without 0.05 g of the marine supplement.
*** $P < .001$.

Table 3
pH of six different diets incubated with pepsin at pH 2.6[a]

Time	1 hour	2 hours	4 hours	s.e.d	Sig				
pH	5.82[a]	5.87[b]	5.91[c]	0.008	***				
Feed	HFF	High F:C	Low F:C	HCF	Alfa A	SBP	s.e.d	Sig	
pH	5.90[b]	5.90[b]	5.92[bc]	5.99[c]	5.76[a]	5.72[a]	0.025	***	
Treatment		Feed alone		Feed + supplement		s.e.d	Sig		
		5.77[a]		5.96[b]		0.014	***		

HCF, high-cereal feed; HFF, high-fiber feed; High F:C, 70:30 fiber:cereal mixture; Low F:C, 30:70 fiber:cereal mixture; SBP, Alfa A and sugar beet pulp; s.e.d, standard error of difference; Sig, significance level.
[abcd]Values in the same row not sharing common superscripts differ significantly ($P < .001$).
[a] Table compares pH of six different fiber and concentrate diets incubated in vitro with a pepsin HCl solution of pH 2.6 for 4 hours with (+) or without 0.05 g of the marine supplement.
*** $P < .001$.

Table 3 shows the pH across time, feeds, and treatment when incubation was carried out a in solution of pepsin HCl adjusted to pH 2.6 for 4 hours. The pH across all feeds rose ($P < .001$) from the first measurement at 1 hour to 4 hours after inoculation.

Alfa-A and SBP produced the lowest pH values, whereas the Low F:C mixture and the high-cereal feed (HCF) alone produced the highest pH of ≥5.9. The high-fiber feed (HFF) and the high F:C mixture showed intermediate results at a pH of approximately 5.9.

Across all time periods and feeds under strong (pH 2.6) acidic conditions, the addition of the marine supplement raised the pH by almost 0.2 ($P < .001$), indicating a small but effective buffering action.

3.2. Experiment 2

Gas production profiles of the recorded cumulative gas volumes, together with the fitted [22] curves from the four incubated feeds for 68 hours, are shown in Fig. 1. The most gas was produced from the high-cereal feed (HCF), while the least gas was from the high-fiber feed (HFF). The addition of the supplement only marginally increased the amount of gas produced in all diets. The exception was the high-cereal diet in which the feed without the supplement produced more gas. However, none of these differences was significant. This effect can be more clearly seen in Fig. 2, where the milliliter of gas produced per hour was increased after 10 hours of incubation by the addition of supplement to all the feeds except for the high-cereal feed (HCF).

Table 4 shows that at the end of the 68-hour incubation period, the only difference ($P < .05$) in all parameters measured was for lag time. The addition of supplement reduced the lag time across all feeds by an average of 0.6 hour.

However, supplement addition did not affect endpoint amount, rate, or extent of substrate degraded, nor did it affect the amount of lactate produced or the pH of the postferment digesta.

Differences ($P < .05$) were noted, however, between feeds with the addition of high-cereal feed (HCF) increasing the Y95, FRGP, L_T, DM loss, and Ext Deg (Table 4). The time to reach 50% and 95% of the total gas produced and the pH of the post ferment-solution was less ($P < .05$) when the HCF alone or the Low F:C mix was present compared with the HFF or the high F:C mix.

4. Discussion

4.1. Experiment 1

A pH gradient exists in the equine stomach, ranging from 5.4 in the fundic region to 1.8 in the pyloric region [23]. In order to represent digestive and fermentative activity in the more acidic areas of the equine stomach, a range of diets were incubated at pH 4.1 and 2.6 for a total of 4 hours.

The reduction of acidity across the range of fiber and concentrate feeds by the addition of the supplement at only 1% of the total feed demonstrated a buffering action at both pH 4.1 and pH 2.6 over a 4-hour period. The increase in pH of almost 0.2 (0.17 and 0.19 respectively) indicates that the supplement was equally effective over a range of conditions. These results indicate that the supplement is worthy of further in vivo investigations. For example, should the moderate buffering activity observed in this experiment occur in vivo, it is possible that the supplement could be used as an EGUS management tool. Successful treatment of EGUS with omeprazole paste is based on inhibition of gastric acid secretion [24] and a subsequent increase in pH. Maintenance of a slightly higher pH, without affecting gastric acid secretion, may prevent initiation of ulceration and render treatment unnecessary; however, further in vivo tests are required to confirm this.

Occurrence of the higher pH values noted for the cereal-based feeds and the lower pH for the fiber feeds are opposite to what is commonly observed in vivo. The methodology applied in this experiment was limited to the measurement of the effect of acid hydrolysis and pepsin activity only and did not mimic inoculation with saliva or normal fundic in vivo fermentation. It is likely, therefore, that the high-fiber feed (HFF), including Alfa-A and SBP, remained relatively intact during the incubation period. A previous study has shown that the protein in SBP is not released for absorption until fiber degradation occurs in the hindgut [25]. This means that the potential buffering action

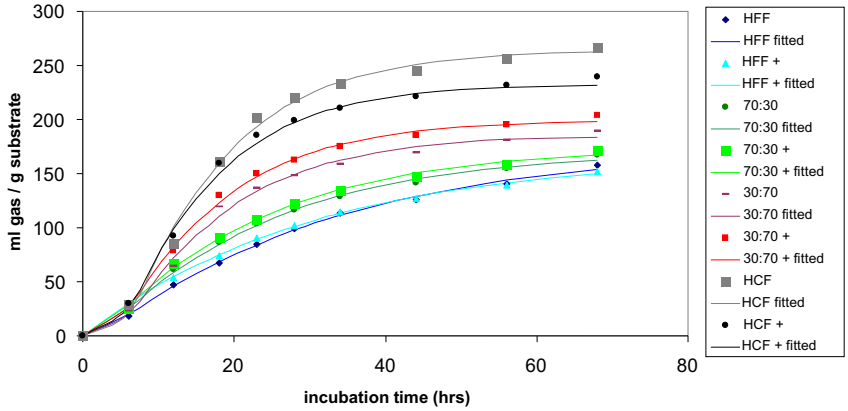

Fig. 1. Cumulative gas production profiles (mL of gas/g of substrate) for high-fiber feed (HFF), high fiber:cereal (70:30) ratio, low fiber:cereal (30:70) ratio, and high-cereal feed (HCF) when incubated in vitro with equine fecal inoculum with (+) or without supplement. Each value represents the mean of two bottles, while the line indicates the profile model as described by France et al [22].

from protein and calcium, the latter associated with the pectic fraction of the cell walls, would not have been released by the action of HCl or by pepsin. However, cereal feeds are susceptible to stomach fermentation [26] and acid hydrolysis, so the more digestible high-concentrate feed could have released the alkaline minerals Ca, K, and Mg, which would have increased the pH of the mixture and thus explain the higher pH values noted for these feed combinations.

While an in vitro methodology using HCl and pepsin may be sufficient for determining buffering capacity of a feed supplement, further development of an in vitro system that accurately mimics inoculation with saliva and fundic fermentation is required if in vitro methods are to be used to measure the breakdown of feed within the equine stomach.

4.2. Experiment 2

The in vitro gas production system of Theodorou et al [20] has been used widely to measure the rate and extent of dry matter disappearance of diets commonly fed to horses [27], as well as the influence of particle size [28] and the effect of additives [29] on fermentation kinetics of a wide variety of horse feeds.

Gas production profiles of the high-fiber feed and the high-concentrate feed reflect the potential degradability of each feed. The addition of the supplement did have a slight overall positive effect on gas production profiles (which, in the in vitro gas production technique, is indicative of an increase in the extent of degradation) for the high-fiber and mixed fiber:cereal diets but did not show this effect with the high-cereal diet. It is not known at this time whether an increase in gas production as noted in vitro is predictive of potential beneficial in vivo effects (due to increased digestibility of high-fiber feeds) or whether some of the reported side effects of feeding highly digestible starches such as gas colic or even laminitis [13] will become significant considerations (due to increased gas production) when the product moves to in vivo testing. The beneficial effect of the supplement seems to be most pronounced in the first 20 hours of incubation, with even the high-cereal diet showing small increase in degradation, indicated by the increase in production (Fig. 2) positive

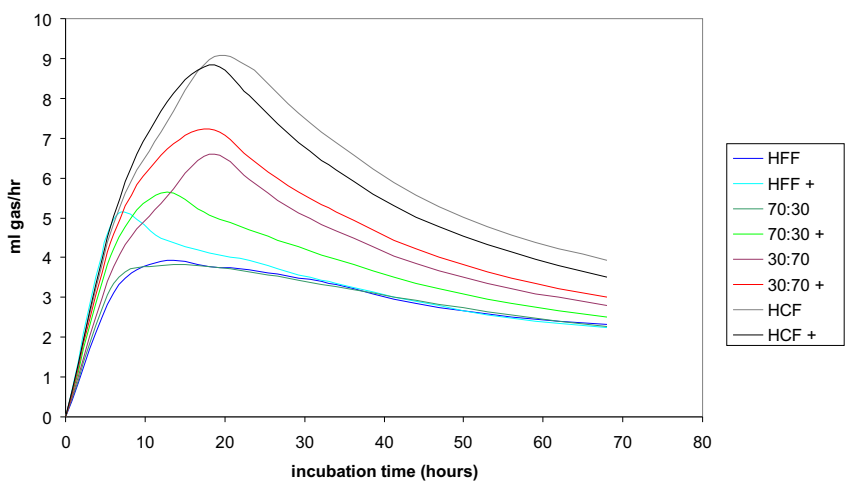

Fig. 2. Milliliters of gas produced per hour from high-fiber feed (HFF), high fiber:cereal (70:30) ratio, low fiber:cereal (30:70) ratio, and high-cereal (HCF) feeds when incubated with equine fecal inoculum with (+) or without supplement.

Table 4
Degradation measurements from feeds incubated with an equine fecal inoculum for 68 hours[a]

Feeds						Treatment	
	HFF	High F:C	Low F:C	HCF	s.e.d	No Supplement	+0.02 g Supplement
Y95	161.8[a]	163.4[a]	182.8[a]	236.5[b]	9.70	188.0	184.3
FRGP (mL/h)	0.033[a]	0.040[b]	0.050[c]	0.043[bc]	0.0031	0.041	0.041
Lag (h)	0.64[a]	1.35[b]	3.46[c]	4.17[d]	0.193	2.7[b]	2.1[a]
T 50	22.4[c]	19.1[b]	14.6[a]	14.3[a]	1.07	18.0	17.2
T 95	90.7[c]	67.7[b]	44.9[a]	41.6[a]	1.8	61.5	61.0
DM loss (g/kg)	510[a]	542[b]	605[c]	658[d]	11.01	579	579
Ext deg	24.39[a]	28.16[b]	35.55[c]	39.02[d]	1.007	31.40	32.16
Lactate (mmol/L)	1.04	1.25	1.41	1.10	0.280	1.25	1.15
pH	6.72[b]	6.67[b]	6.61[a]	6.59[a]	0.027	6.66	6.63

DM, dry matter; FRGP, fractional rate of gas production; HCF, high-cereal feed; HFF, high-fiber feed; High F:C, 70:30 fiber:cereal mixture; Low F:C, 30:70 fiber:cereal mixture; SBP, Alfa A and sugar beet pulp; s.e.d, standard error of difference; T 50, time to produce 50% of total gas; T 95, tie to produce 95% of total gas.
[abcd]Values in the same row with different superscripts are significantly ($P < .05$) different.
[a] Table compares feed degradation measurements from four fiber and concentrate feeds incubated with an equine fecal inoculum for 68 hours in an in vitro gas production system.

effects on gas production, although this was reversed later on in the fermentation process. This positive effect on fermentation is most likely caused by the addition of scFOS, which has been previously shown [17] to stimulate the degradation of fiber feeds in vivo in horses. The fact that the scFOS did not have any positive effect on the degradation of the high-concentrate feed is also in agreement with previously recorded in vivo data and may be attributed to the fact that cereals have a positive effect on cellulolytic activity in the hindgut [30]. High-fiber diets do not have the same stimulatory effect (on the microbial population) [30], and therefore, the scFOS worked most effectively with the fiber feeds.

The fact that endpoint degradation levels were similar between the supplemented and nonsupplemented diets is most likely a function of continued microbial action on the fibrous portion of the diets. Concentrate feeds are a mixture of rapidly degraded starch (endosperm of the grain) and poorly degraded fibrous material (cell walls, i.e., hull and husk); the starch would degrade rapidly, leaving the poorly degraded lignified cell walls which even the addition of a fermentation stimulant could not improve. Moreover, the degradability of the scFOS would mean that it disappeared earlier in the incubation process and thus could no longer stimulate fermentation. The mean retention time of digesta in the gastrointestinal tract of the horse has been recorded to be between 30 and 40 hours [25] in ponies fed a variety of fiber and concentrate diets. Results recording degradation after 40 hours are biologically meaningless for the horse, but incubations times were extended to facilitate accurate mathematical modelling.

The rapid fermentation rate and extent of feed degradation, which increased as the concentrate proportion increased, is reflected in the final pH of the solutions after 68 hours of incubation, which were more ($P < .05$) acidic for the HCF and Low F:C mixture than for the HFF and the High F:C mixture diets.

5. Conclusions

The main limitations of a study of this nature center on the lack of in vivo efficacy data. However, preliminary results that indicate efficacy, such as are reported here, provide sound rationale for progression to future in vivo studies. These could include documentation of the magnitude and duration of gastric buffering effects and a thorough investigation into the effects of the supplement on hindgut microbial flora.

In summary, the results of these experiments show that the addition of a marine-derived, multimineral supplement to a range of fiber:concentrate diets had a positive ($P < .001$) buffering effect during in vitro foregut digestion and slightly stimulated early fermentation rate in in vitro hindgut conditions.

Acknowledgments

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