



In vitro assessment of various Leiber YeaFi[®] products on the fermentation of high-starch and high-fibre substrates in horses

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As a hindgut fermenter (or 'large intestine fermenter'), horses are dependent on the supply of crude fibre in the daily feeding. In the large intestine, especially in the caecum, there are large quantities of cellulolytic bacteria that break down cellulose and hemicellulose into volatile fatty acids such as acetic acids and propionic acids. They contribute significantly to the energy supply of the horse. Glycogen reserves in the muscle of horses are limited, so that the production of volatile fatty acids (VFA) significantly contributes to the horse's energy supply. According to ZEYNER (1995), 30-40% of the energy requirement in maintenance metabolism can solely be ensured via colon activity. Consequently, impairments to the colon activity always lead to bottlenecks in the daily energy supply. The preservation and promotion of intestinal health, especially the promotion of colon activity, by an intact and active microflora, plays a vital role for horses. Heavy meals, such as very high-fibre or very high-fibre or lignin-rich rations, can lead to imbalances in the microflora.

The experiment described below was set up to study the impact of various brewers' yeast fibre products (Leiber YeaFi[®]) on the fermentation of high-starch and high-fibre substrates in an in vitro system.

Material and Methods:

Leiber YeaFi[®] products are manufactured in a special processing technique and combine the known positive digestive physiological properties of brewers' yeast (PBY) with those of different high-fibre carriers, such as spent grains, beet pulp or apple pomace. The carrier substrates differ not only in terms of their crude fibre content, but especially in their fibre structure, i.e. the content of soluble and insoluble fibres such as hemicellulose, cellulose and pectin. Table 1 shows a list of Leiber YeaFi[®] products used in the experiment.

The experiment was carried out with the in vitro gas production technique of THEODOROU et al. (1994), which was inoculated with 'equine faecal inoculum'. The use of equine faeces instead of a caecum material, as inoculum has previously been studied by JULLIAND et al. (2005), but also by MOORE-COYLER et al. (2002) and MURRAY (2005). No significant differences were found. Faeces thus represents a suitable material for the representation of colonic digestibility in an in vitro system.

For each **Leiber YeaFi**[®] product (see Table 1), gas production (GP) was measured every 10 minutes over a period of 72 hours. The differences in the intensity of microbial degradation in the caecum and colon of substrates containing, for example,

a high content of easily fermentable carbohydrates was studied by KÖPKE (2009). In this study, she compared various 'in vitro techniques' among each other and found that fermentations can be illustrated very well with gas formation. The gas production (GP) provides a good estimate of the degradation rate of raw materials. According to KÖPKE (2009), a temporal representation of the fermentation rate using GP is particularly important in horses, since the passage through the gastrointestinal tract is relatively guick compared to ruminants. An increased degradation rate in the colon thus not only has a positive impact on the digestive system in general, but in particular on the degradation and remodelling processes of the microflora and thus on the formation of VFA (volatile fatty acids), resulting in better energy supply (e.g. propionic acid) and improved intestinal health (e.g. butyrates). The measurement of VFA contents and the pH was carried out once at the end of the experiment.

The test was carried out with two different nutrient substrates: a high-fibre substrate (HF), consisting of (grass) hay and alfalfa in a 70:30 ratio and a high-starch substrate (HS), consisting of (grass) hay and a starchy cereal mixture, also in a 70:30 ratio.



Table 1: Leiber YeaFi® Products

Name	Description
PBY	100% pure brewers' yeast
BT	40% brewers' yeast bound to 60% spent grains (Leiber YeaFi® BT)
BTR	40% brewers' yeast bound to 30% spent grains (Leiber YeaFi® BTR)
AB	40% brewers' yeast bound to 30% apple pomace* and 30% beet pulp** (Leiber YeaFi® AB)
AC	40% brewers' yeast bound to 30% apple pomace* and 30% carrot pomace (Leiber YeaFi® AC)
AP	40% brewers' yeast bound to 30% apple pomace* and 30% pea fibre (Leiber YeaFi® AP)

* apple pomace depectinized ** sugar beet, unrefined

The above referenced substrates were ground and mixed with the **Leiber YeaFi**[®] products (see Table 1) in accordance with the recommended daily dose of 250g per day and horse (600kg body weight) and added to the system. The pure brewers' yeast (**PBY**) served as a positive control within the experiment.

Results:

The addition of pure brewers' yeast (**PBY**) increases gas production (GP), especially in high-starch rations. Even in high-fibre rations, the GP can initially be raised, but weakens significantly in the further course (see Figure 1). The pH value remains in the ideal range throughout the trial period and even tends to increase.

Fig. 1: In vitro gas production of high-fibre (HF) and high-starch (HS) substrates incubated with 100g brewers' yeast (**PBY**) per day/horse



Leiber YeaFi[®] BT the pure brewers' yeast is bound to 60% spent grains. Compared to all other Leiber YeaFi[®] products, Leiber YeaFi[®] BT contains more protein and fat content and has a closer ratio of soluble and insoluble fibres. For Leiber YeaFi[®] BT, only a small effect on gas production was discovered during the experiment (see Figure 2).

Fig. 2: In vitro gas production of high-fibre (HF) and high-starch (HS) substrates incubated with 250g **Leiber YeaFi® BT** per day/ horse



An increase in the proportion of soluble fibre components in the product, for example, beet pulp as in Leiber YeaFi[®] BTR (see Figure 3), significantly improves the digestibility of spent grains. In the experiment, Leiber YeaFi[®] BTR demonstrated a significant increase in GP in high-starch diets. This result was higher than in Leiber YeaFi[®] BT (see Figure 2) and higher than with the addition of pure brewers' yeast (see Figure 1). After just 10 hours, Leiber YeaFi[®] BTR shows a faster degradation rate (GP: 80ml) in high-starch and high-fibre rations than pure brewers' yeast (GP: 60ml). In the further course, however, the GP drops noticeably with high-fibre rations. At the same time the pH increases, both in high-starch and in high-fibre rations, in comparison to no additive.



Fig. 3: In vitro gas production of high-fibre (HF) and high-starch (HS) substrates incubated with 250g Leiber YeaFi $^{\circ}$ BTR per day/horse



The fibre analysis of **Leiber YeaFi®** AB shows similarly high levels of insoluble and soluble fibres, such as **Leiber YeaFi®** BTR. However, due to the high proportion of apple pomace (30%) and beet pulp (30%) in **Leiber YeaFi®** AB, the content of hemicelluloses in the product increases significantly.

A significant increase in gas production (GP) in high-starch was found, which was even higher in high-fibre diets (see Figure 4). The GP in high-fibre rations increases as much as with high-starch rations without additive (CtrI-HS). At the same time, **Leiber YeaFi®** AB has a very positive effect on the pH in high-fibre (pH 6.7) and, even more so, in high-starch (pH 6.6) rations.



Fig. 4: In vitro gas production of high-fibre (HF) and high-starch (HS) substrates incubated with 250g Leiber YeaFi® AB per day/

Compared to the other brewers' yeast fibre products, adding carrot pomace to **Leiber YeaFi®** AC increases the proportion of soluble fibre constituents and thus also the content of readily fermentable substances.

This is also evident in an increase in GP, both in the high-starch and the high-fibre rations (see Figure 5). The fermentation of high-fibre rations compared to **Leiber YeaFi® AB** (see Figure 4), supplemented with **Leiber YeaFi® AC**, was further improved. The GP in high-fibre rations (HF + AC) with additive is just as high as in the high-starch ration without additive (HS).

Fig. 5: In vitro gas production of high-fibre (HF) and high-starch (HS) substrates incubated with 250g Leiber YeaFi® AC per day/ horse



Leiber YeaFi[®] AP contains a similar amount of insoluble fibres as Leiber YeaFi[®] AB. However, the cellulose content is significantly higher due to the pea fibres (30%). There is a significant increase in GP in both high-starch and highfibre rations (see Figure 6). This effect is similar to Leiber YeaFi[®] AC (see Figure 5) and stronger than Leiber YeaFi[®] AB (see Figure 4).



Fig. 6: In vitro gas production of high-fibre (HF) and high-starch (HS) substrates incubated with 250g **Leiber YeaFi®** AP per day/ horse



During the in in vitro test, samples were taken once from the final substrate for further analysis of the volatile fatty acids (VFA). Adding pure brewers' yeast exhibits a significant increase in VFA production in high-starch diets (Total VFA + 6%), and high-fibre diets (Total VFA + 9%). In particular, butyrates (+ 12%) and propionates (+ 11%) are formed. The brewers' yeast fibre products (**Leiber YeaFi**[®]) are also positive for VFA production, especially in the formation of propionate. Especially the high-starch ration exhibits a regulatory effect on the formation of propionate with simultaneous pH stabilization or increase. **Leiber YeaFi**[®] AP has the highest VFA increase in both high-fibre and high-starch rations – especially in the production of acetate and butyrate.

Discussion:

Celluloses, hemicelluloses and pectins, according to MEYER & COENEN (2014), pass largely unchanged through the small intestine of the horse. The microbial digestion and decomposition into volatile fatty acids (VFA) only take place in the large intestine, which then pass through the intestinal wall into the blood to provide energy supply. ZEYNER (1995) also points out that the energy requirement of a horse can be met by the VFA formed from difficult-to-digest structural substances, which are easily fermented in the large intestine, such as celluloses.

Furthermore, according to ZEYNER (1995), the microbial activity in the large intestine can be promoted in a targeted manner, thereby increasing energy production. Among other things, the composition of the ration or, more precisely, the quantities and qualities of roughage and concentrates have a major influence on the levels of volatile fatty acids (VFA) formed in this process. Traditionally, high-starch rations are often fed to the animals to cover the increased energy requirements, e.g. in sport horses. An oversupply of concentrates can lead to a high content of easily digestible carbohydrates in the colon. This can decrease the content of produced acetic acids (acetate) while the content of propionic acid (propionate) increases. At the same time, the pH decreases (hyperacidity) and the risk of an imbalance in the microflora increases (dysbiosis). CRANDELL (2016) also described the negative impact of dysbiosis in the gastrointestinal tract on common diseases such as colic and laminitis in horses. The cellulolytic bacterial population can die; endotoxins are released, increasing the risk of laminitis. According to ZEYNER et al. (2005), substrates in 'in vitro systems', such as starch, are directly supplied to the large intestine, while in practice starch should already be precaecally digested and therefore not enter the large intestine. The above referenced experimental results regarding the Leiber YeaFi® products for VFA production must therefore be interpreted as very positive, especially in light of an oversupply of starch in the large intestine and the resulting negative consequences such as a dysbiosis or the risk of hyperacidity (pH). However, they can only serve as an initial assessment and should be re-examined in vivo.

The present results for gas production provide a good assessment of how much the different Leiber YeaFi® products can influence the degradation rate and thus the fermentation of high-fibre or high-starch rations in the colon of horses. ZEYNER et al. (2005) discovered in an in vitro experiment that incubation of slightly soluble carbohydrates, as opposed to incubation of pure cellulose, resulted in greater gas formation. According to KÖPKE (2009), an increased degradation rate in the large intestine would not only have a positive impact on the digestibility of the added substrates in general, but also on the degradation and transformation processes of the microflora. Ultimately, therefore, it has a positive impact on the formation of VFA (volatile fatty acid) for improved energy supply (e.g. propionate) or improved intestinal health (e.g. butyrate) of horses. Especially in high-starch diets, the pH value should remain within the ideal range so as not to adversely affect the microflora.

The tested **Leiber YeaFi**[®] products showed very positive results in gas production (see Table 2) as well as in pH value and VFA production. The amount and course of the GP as well as VFA profiles differed within the products corresponding to their fibre profile: from high content of easily soluble fibres (e.g. **Leiber YeaFi**[®] **AC**) to high levels of insoluble fibres in **Leiber YeaFi**[®] **AB** (esp. hemicelluloses) and **Leiber YeaFi**[®] **AP** (esp. cellulose).



 Table 2: Impact of various Leiber YeaFi[®] products on gas production (GP) in high-starch and high-fibre substrates

	Nutrient substrate			
	high-fibre		high-starch	
Product	Course	Level	Course	Level
pure brewers' yeast (PBY)		-		
Leiber YeaFi® BT	-	-	-	-
Leiber YeaFi [®] BTR	-			
Leiber YeaFi® AB	A	A	A	
Leiber YeaFi® AC				
Leiber YeaFi® AP				

▲ Increase ▼ Decrease - No impact

MEYER & COENEN (2014) already described in their book on horse feeding that adding protein-rich components such as pure brewers' yeast to low-protein such as straw can significantly increase their microbial digestion. The positive effect of brewers' yeast on microbial digestion can also be seen in the experiment, especially in high-starch diets (see Figure 1). By combining brewers' yeast with different fibres (Leiber YeaFi[®] products), its positive effect is significantly improved. The addition of Leiber YeaFi® AB (see Figure 4), Leiber YeaFi[®] AC (see Figure 5) and Leiber YeaFi[®] AP (Figure 6) significantly increases gas production in high-starch and, above all, high-fibre rations. The increase is more pronounced than with the addition of brewers' yeast (PBY) alone and also stronger than in high-starch rations without additive. GARBER et al. (2016) also noted that high-fibre rations have the potential to provide more energy than starch-rich rations when supplemented with yeast.

Leiber YeaFi[®] products can thus be used in a targeted manner in high-starch rations to counteract hyperacidity by stabilizing the pH with simultaneous regulation of VFA production. Furthermore, Leiber YeaFi® can increase the production of VFA in basic feed to the extent that it provides similar energy levels for the horse as in a high-starch feed. High-fibre feeding combined with Leiber YeaFi® can thus provide enough energy to substitute sugar and starch content in the ration, e.g. in metabolic disorders such as EMS (equine metabolic syndrome) or as part of diet feed to reduce body weight. In addition, a weight loss diet would be supported by the high levels of insoluble fibrous components such as cellulose in Leiber YeaFi[®] AB or Leiber YeaFi[®] AP. Cellulose simulates the feeling of being full in the digestive tract and thus provide improved feeling of satiety as well as improved peristalsis. This is particularly beneficial for horses that are supposed to be fed rather restrictive or calorie-reduced diets.

Leiber YeaFi[®] products offer even more benefits for the horse. The type and amount of crude fibre is crucial, for example, when it comes to how well the horse can store water

and electrolytes. They are absorbed like a sponge by cellulose and hemicellulose in the large intestine and are then available when needed. If the endogenous water and electrolyte reservoir is too low, life-threatening disorders loom in case of high sweat loss. **Leiber YeaFi**[®] products have an increased ratio of crude fibres with different levels of celluloses (e.g. **Leiber YeaFi**[®] AP) and hemicelluloses (e.g. **Leiber YeaFi**[®] AB and AC) and therefore can certainly make a valuable contribution to the water and electrolyte balance.

Summary:

The addition of brewers' yeast increases gas production, but also the production of volatile fatty acids (VFA), especially in high-starch rations. This effect is less pronounced in highfibre rations. By combining brewers' yeast with different fibre carriers in Leiber YeaFi® AB, Leiber YeaFi® AC and Leiber YeaFi® AP significantly increases gas production in highstarch, but above all, high-fibre rations. The GP increase is more pronounced than with the addition of brewers' yeast (PBY) alone and also stronger than in high-starch rations without additive.

All **Leiber YeaFi**[®] products exhibit a positive effect on VFA production during the experiment, as well as a stabilizing effect on the pH, especially in high-starch rations. They could thus counteract, for example, hyperacidity resulting from starchrich feeding. Of particular interest, however, are the results regarding high-fibre feeding. In this case, an improvement in digestibility directly leads to an improved energy supply for the horse due to increased VFA production. Especially horses with maintenance metabolism, but also horses with chronic metabolic disorders such as EMS, which are mainly fed high-fibre, low-calorie diets, could benefit from the use of **Leiber YeaFi**[®] products.



Leiber YeaFi® product features



"In-vitro-Beurteilung verschiedener Leiber YeaFi® Produkte auf die Fermentation von stärke- und faserreichen Substraten beim Pferd" University of Glasgow, College of Medical Veterinary and Life Sciences; Prof. Dr. J.-A. MURRAY (2018), unpublished

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