PROFEED®

AN INTESTINAL ACTION
FOR BETTER HORSE HEALTH

PROFEED® - AN INTAKE OF PREBIOTIC SOLUBLE FIBRES

PROFEED® supplies soluble fibres modulating the microbiota and the intestinal activity, with benefits for the host.

Short-chain fructo-oligosaccharides (scFOS) are ingredients that are found naturally in small amounts in fruit and vegetables such as bananas, wheat, onions, and asparaguses.

PROFEED® is made up of scFOS obtained from beet sugar following an enzymatic bioconversion of sucrose leading to the formation of fructans composed of one glucose molecule linked to 2-4 fructose molecules by a ß (2-1) linkage.

PROFEED® is definitely and constantly composed of the three types of components GF2, GF3, and GF4.

ScFOS are not hydrolysed by mammalian digestive enzymes, but are selectively fermented by some bacteria in the intestinal microbiota.

Those kinds of soluble fibres will be preferentially used by beneficial bacteria such as *Lactobacilli* strains.

ScFOS are defined as prebotics [2]. Therefore, the intake of PROFEED® allows modulating the composition and activity of the intestinal microbiota that is beneficial to the host.

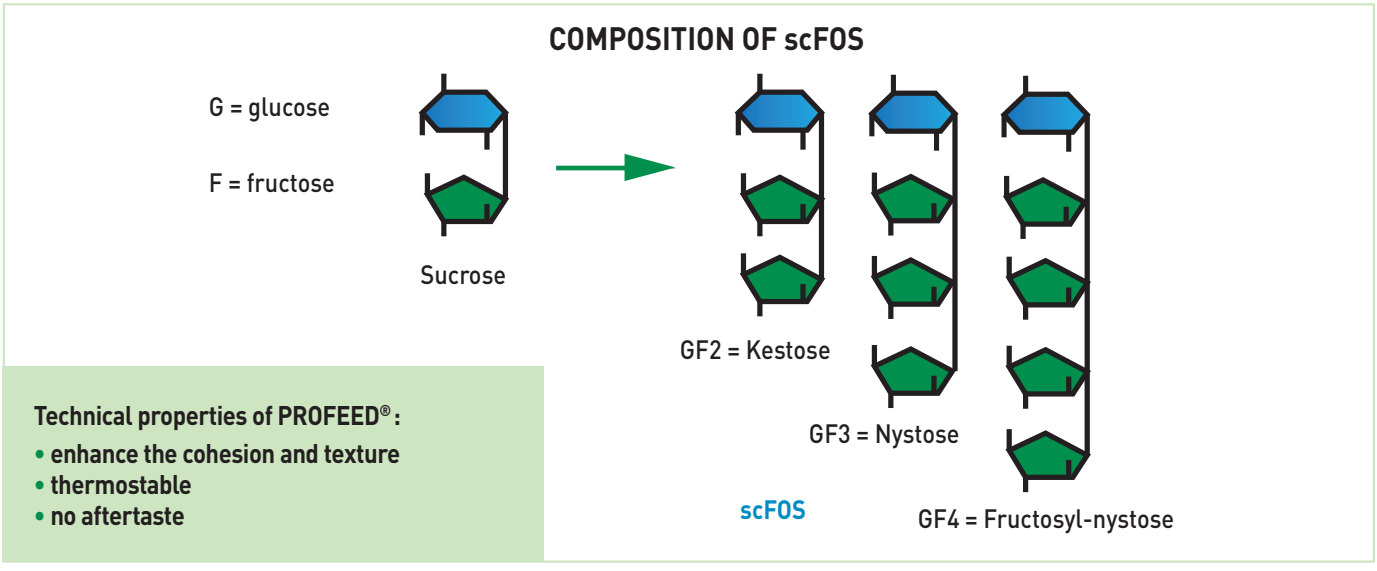

Figure 1: Molecules of scFOS
For more than 20 years, many scientific studies and field trials have confirmed the benefits of PROFEED®, for many animals such as poultry, rabbits, pigs, horses, calves, dogs, cats, as well as fishes.

MICROBIOTA - A KEY ELEMENT FOR HORSE HEALTH

Various microorganisms live in the whole gastrointestinal tract of horses.

Horses are herbivores classified as hindgut fermenters, meaning their digestive and metabolic systems adapt to use fibres. Notably, the voluminous hindgut contains a significant number of cellulolytic bacteria. Various microorganisms, in large quantities, live in the gastrointestinal tract, from stomach to colon; and interact with each other (Table 1). The abundance, diversity, and activity of microorganisms differ between the different regions of the gastrointestinal tract, resulting in different utilisations of dietary starch and fibres.

- Dietary fibres are mainly fermented in the hindgut, especially in the caecum, producing short chain fatty acids (SCFA).
- In horses, dietary starch use implies digestion by a pancreatic enzyme and fermentation by the microbiota. The starch is used from the stomach to the colon: a part of starch is fermented by several bacteria species in the stomach and in the small intestine, leading to the formation of lactate and SCFA [2]. The other part of starch is hydrolysed by pancreatic α-amylase as in other non-ruminant species. Finally, after reaching the hindgut, starch will be fermented by amylolytic bacteria, leading to the formation of SCFA and lactate.

Table 1: Gastrointestinal microbiota in adult horses (from [3] and [4])

Segment of the digestive tract	Total anaerobic bacteria, cfu/mL	Main entities	Main digestive functions	Main end-products
Stomach	10^9	*Lactobacilli, Streptococci* Lactate-utilizing bacteria	Utilisation of starch and highly fermentable carbohydrates	Lactate SCFA
Small intestine	10^6 to 10^9	*Lactobacilli, Streptococci, Enterobacteria,* Enterococci, Lactate-utilizing bacteria	Utilisation of starch and highly fermentable carbohydrates	Lactate
Hindgut *Caecum* *Colon*	10^7 to 10^9 10^9	Cellulolytic bacteria *Glycolytic/amylolytic* bacteria (*Streptococci, Lactobacilli)* Lactate-utilizing bacteria	Utilisation of fibres Utilisation of starch	SCFA Lactate

Starch influences the microbiota activity and impacts digestive function and health.

A high-starch diet can result in digestive disorders. Indeed, the capacity of horses to hydrolyse starch is limited because of low availability and activity of their pancreatic α-amylases [5]. Consequently, when increasing the quantity of starch in a diet, there is a risk to exceed the small intestine enzymatic capacity to hydrolyse starch. Then, greater amount of non-degraded starch will reach the hindgut where it will be rapidly fermented (Figure 2; [6]).

Rapid starch fermentation disturbs the microbial and pH balance in the caecum and in the colon. *Lactobacillus spp* and *Streptococcus spp* proliferate whereas the amount of cellulolytic bacteria decreases [7].

Besides the microbiota modification, acid production increases [7];[6]. Accumulation of acids, especially lactic acid, overpowers the hindgut buffering capacity, resulting in lower caecal pH. A low pH is associated with a modification of the osmotic pressure, a proliferation of undesired bacterial

populations, a lysis of cellulolytic bacteria and, finally, with an increasing risk of colic and laminitis [8].

Due to the horse limited capacity to hydrolyse starch, it is recommended to avoid any sudden diet changes or any carbohydrate overload. Nevertheless, athlete horses often need high levels of starch sometimes leading to an inappropriate concentrate/forage ratio. In such situations, dietary practices are in conflict with digestive physiology of horses and can result in digestive disorders. A daily starch intake of more than 2% body weight/day increases the amount of starch reaching the hindgut, which can have a negative effect on the microbial fibrolytic activity, affect fibre degradation, and lead to digestive disorders [9]. Thus, it is crucial to have controlled-effects diets, especially when horses are fed with high concentrate diets.

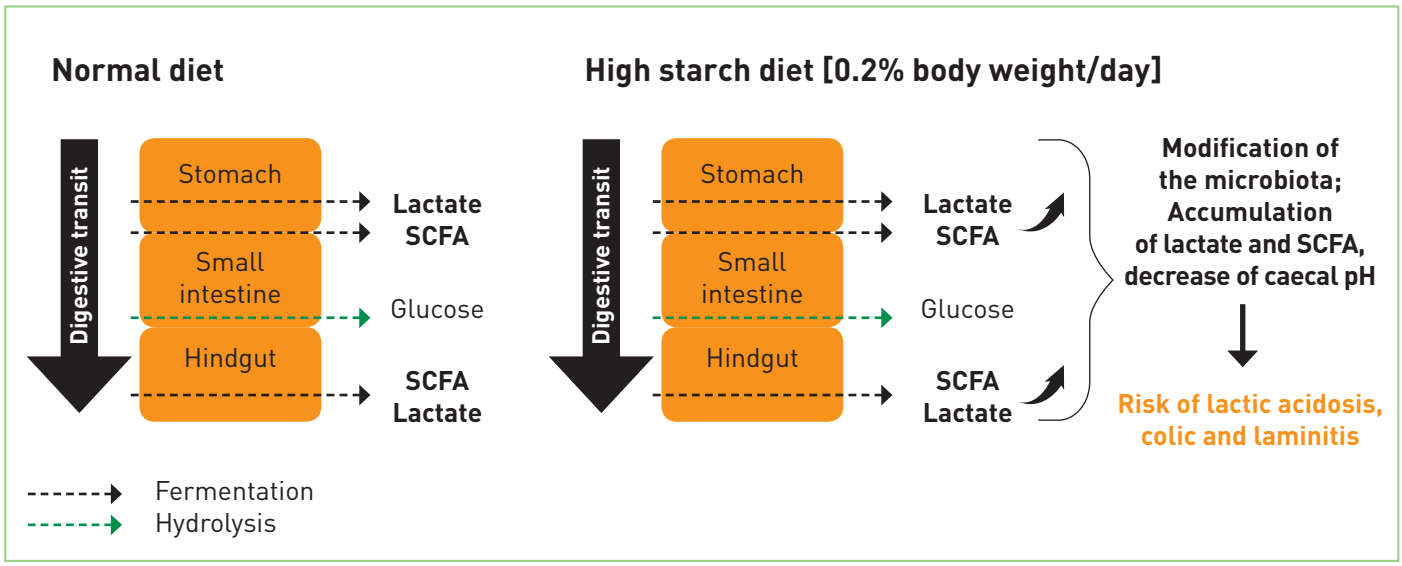

Figure 2. A high dietary level of rapidly fermented starch modifies the microbiota and is a risk factor for metabolic disorders.

Balancing the microbiota can reduce digestive and metabolic disorders and control diets containing high levels of starch in horses. Thanks to its prebiotic properties, 30 g PROFEED® supplementation/day modulate the intestinal microbiota activity in horses.

PROFEED® AND DIGESTIVE SYSTEM - BALANCED MICROBIOTA

PROFEED® balances the microbiota and increases acetate and butyrate production.

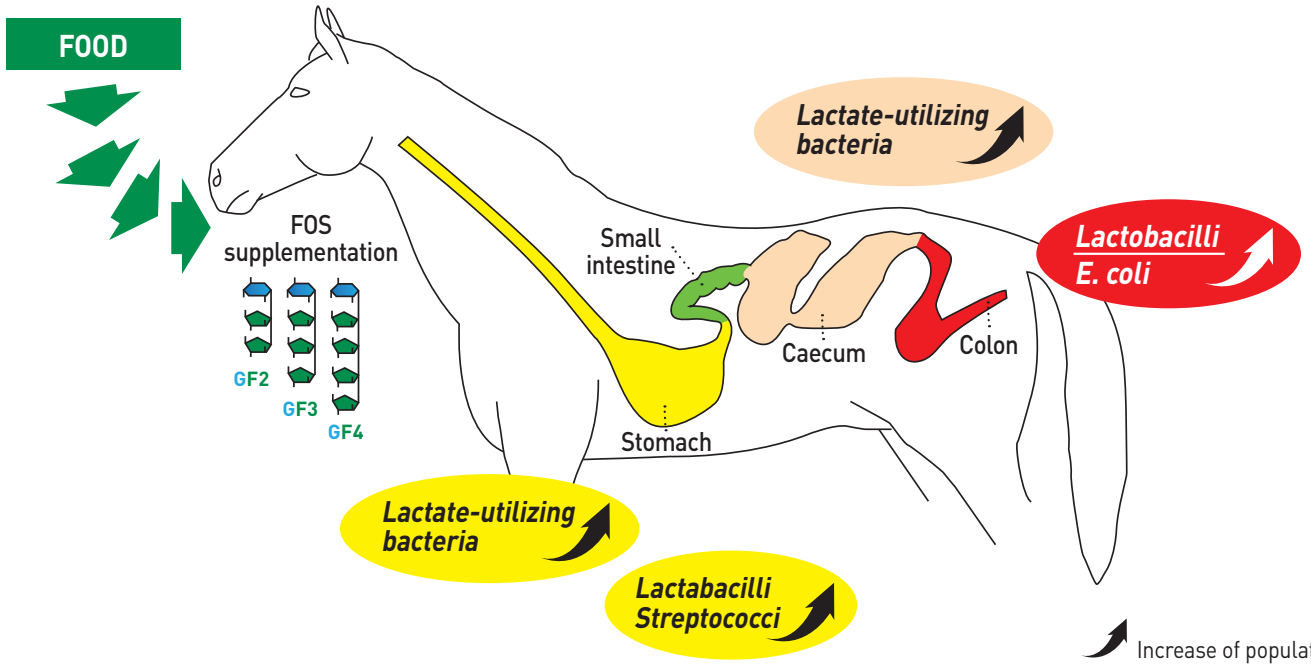

Figure 3. A supplementation with PROFEED® increases lactate-utilizing bacteria in precaecal digestive tract and in the large colon and increases the Lactobacilli/E. coli ratio [1, 10-12].

In horses, the intestinal microbiota is modified when the diet is supplemented with PROFEED® (Figure 3). In the stomach, a supplementation with 30 g PROFEED®/day increases *Lactabacilli*, *Streptococci* and lactate-utilizing bacteria [12]. Supplementation with PROFEED® also affects the colonic microbiota and increases lactate-utilizing bacteria [1].

The microbiota modification by PROFEED® is accompanied by an increase of SCFA, notably acetate and butyrate [10]; [11]; [1, 12]; but neither caecal pH nor D-lactate are modified, which is consistent with the concomitant increase in lactate-producing bacteria and lactate-utilizing bacteria [1, 12].

Thanks to PROFEED®, competitive exclusion of potentially pathogenic bacteria limits the risk of digestive disorders in horses.

A supplementation with PROFEED® limits the risk of digestive disorders in horses. A dose of 0.35% PROFEED® in diets not only significantly decreases the incidence of digestive disorders (53% in the control group and 7% in the scFOS group, n = 15/group), but also their seriousness (Wolter, unpublished data). In humans, a supplementation with scFOS shortens the time required by the microbiota to return to its initial equilibrium when people suffer from diarrhoea and severe microbiota disturbance [15].

An increase of the potentially pathogenic bacteria population is associated with enterocolitis, and severe diarrhoea in horses [11]. A supplementation with PROFEED® results in a competitive exclusion of potentially pathogenic bacteria, included *E. coli*, due to the proliferation of *Lactobacilli* in horses [17], but also in dogs [16]. Indeed, a supplementation with PROFEED® modifies the faecal microbiota by increasing *Lactobacilli/E. coli* ratio in adult horses and yearlings [10]; [11]. Thus, supplementation with PROFEED® reduces the risk of such disease by increasing growth of *Lactobacilli* and by limiting growth of potentially pathogenic bacteria.

PROFEED® AND DIGESTIVE SYSTEM - REDUCED DIGESTIVE DISORDERS

PROFEED® stabilises the microbiota, increases production of SCFA in caecum and prevents D-lactate accumulation in colon when horses are submitted to sudden dietary changes.

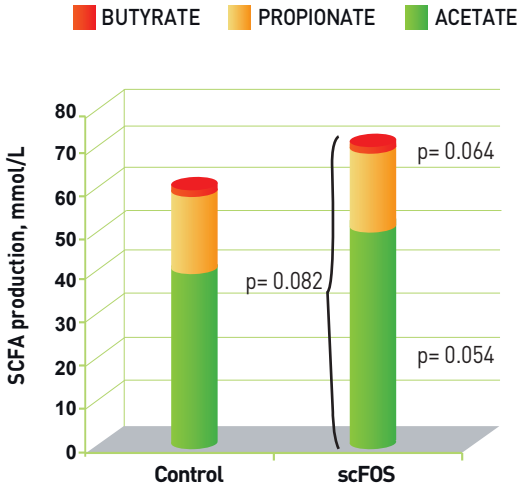

Figure 4. 30 g PROFEED®/day increase caecal SCFA production in horses submitted to a barley challenge [1].

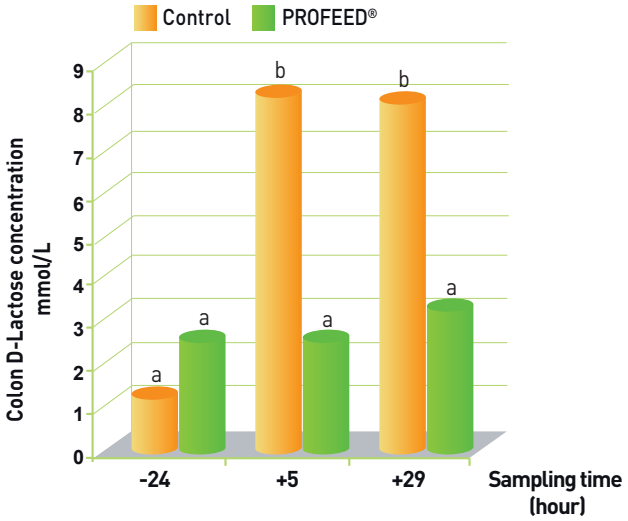

Figure 5. 30 g PROFEED®/day avoid colon D-lactate accumulation in horses submitted to barley challenge [1]. Sampling time is relative to barley challenge. Columns with different superscripts are significantly different (p < 0.05).

A supplementation with 30 g PROFEED®/day stabilises the microbiota, even when horses are submitted to sudden dietary changes. Suddenly adding 0.3% body weight of barley in horse diets increases total anaerobes, lactate-utilizing bacteria, and *Lactobacilli* in colon with scFOS compared to the control group 19 hours before the barley challenge, but those levels remain stable 5 hours and 19 hours afterwards [1]. Resulting in the microbiota stabilisation, SCFA production increases in caecum whereas D-Lactate accumulation is prevented (Figures 4 and 5).

Besides the effect on the colon and the caecum, PROFEED® also modifies the foregut microbiota [12]. Such effect may limit the disruption of microbial populations when increasing starch in diet, notably by enhancing precaecal polysaccharide digestion.

Based on the available data for other species, and in particular for broilers, scFOS are able to increase total amylase activity within the small intestine by increasing *Bifidobacteria* and *Lactobacilli* [14]. In this point of view, scFOS may have a beneficial effect on digestive disorders in the upper part of the digestive tract, like gastric ulcers.

Abrupt changes in horse diet are associated with changes of the microbial population in the digestive tract.
Thus, it may be interesting to stabilise the microbiota to avoid digestive disorders when horses are submitted to sudden dietary changes.

Finally, a supplementation with PROFEED® in high starch diets stimulates precaecal digestion, avoids lactate-producing bacteria proliferation, and D-lactate accumulation that is responsible for decreasing caecal pH. As a result, PROFEED® reduces the risk of digestive disorders.

PROFEED® AND INSULIN SENSITIVITY

PROFEED® modulates glucose tolerance in several species.

By modulating the intestinal microbiota and its fermentative activity, PROFEED® is able to improve insulin sensitivity. Indeed, there is evidence from studies involving insulin-resistant animals, including veal calves fed with a load of lactose [18], obese dogs [19] and more recently obese rodents [20].

PROFEED® increases insulin sensitivity in obese horses.

Like in other species, overweight and adiposity increase fat mass and play a role in modifying insulin sensitivity in horses [21]. This can have important consequences because it increases the risk of laminitis [22]; [23], hyperlipidaemia and osteochondrosis [24], reduces performance, and alters reproduction. Therefore, there is an interest in developing management strategies, including diet changes countering insulin resistance.

Similarly to what is observed in other species, PROFEED® can modulate insulin sensitivity in horses. Eight insulin-resistant Arabian geldings with a body condition score (BCS) of 8/9; receiving 45 g PROFEED®/day for 6 weeks in a 50:50 forage and grain concentrate ration have been included in a study to know if PROFEED® modulates insulin sensitivity [25].

In this experiment, a decrease in basal plasma insulin was observed without modifying plasma glucose concentration (Figure 6). These results are rather comparable to those obtained when a starch and sugar diet is replaced by a fat and fibre diet [26-28].

These modifications occur without changing body weight or body condition score, which suggests the effects of PROFEED® are not mediated by weight loss. Such results were also found in a study involving obese dogs, wherein a 6-week PROFEED® supplementation (1%) was associated with improved insulin sensitivity independent of body weight changes [19].

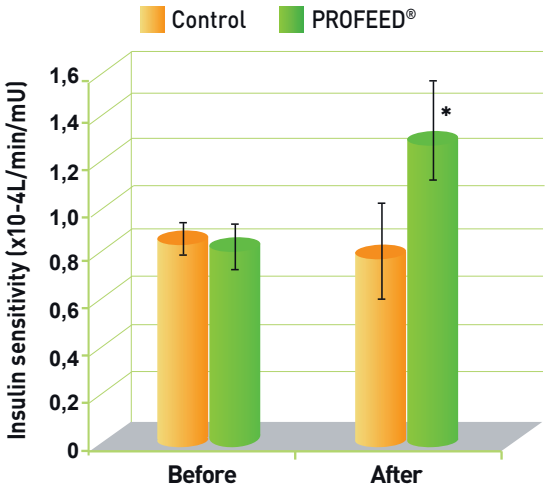

Figure 6. A supplementation with 45 g PROFEED®/day for 6 weeks reduces postprandial insulin sensitivity in obese horses [25].
****: p<0.05 between control and scFOS.***

PROFEED® AND INSULIN SENSITIVITY

Several mechanisms may explain the insulin-sensitizing effect of PROFEED® supplementation.

Gut microbiota composition and activity regulate the host's metabolism notably insulin sensitivity via endocrine and immune signalling systems (Figure 7).

- Greater production of specific SCFA associated with PROFEED® may improve insulin sensitivity via direct effects on insulin signalling mechanism or indirectly via an increase of hormones such as Glucagon-Like Peptide 1 (GLP1), which is associated to enhanced glucose tolerance [29]. Such results are observed in dogs and in mice [30]. Additionally, PROFEED®-induced microbiota modifications alter the production of biliary acids and may thus decrease insulin resistance in diet-induced obesity mice [40].

- Greater production of SCFA associated with PROFEED® may improve insulin sensitivity via effects on inflammatory signalling. In mice, SCFA exert an anti-inflammatory effect [31]. Additionally, changes in the gut microbiota (especially decreased *E. coli*) or mucosal barrier function with scFOS may decrease the absorption of Lipopolysaccharides (LPS) and reduce inflammation and insulin resistance associated with low-grade endotoxemia [32].

These different mechanisms are most likely to be involved in horses. Indeed, insulin sensitivity decreases when the horses receive an intravenous injection of *E. coli* LPS and a grass hay diet [33]. Additionally, scFOS alter hindgut microbiota composition and especially increase the *Lactobacilli/E. coli* ratio and the production of SCFA [10; 11; 12; 1].

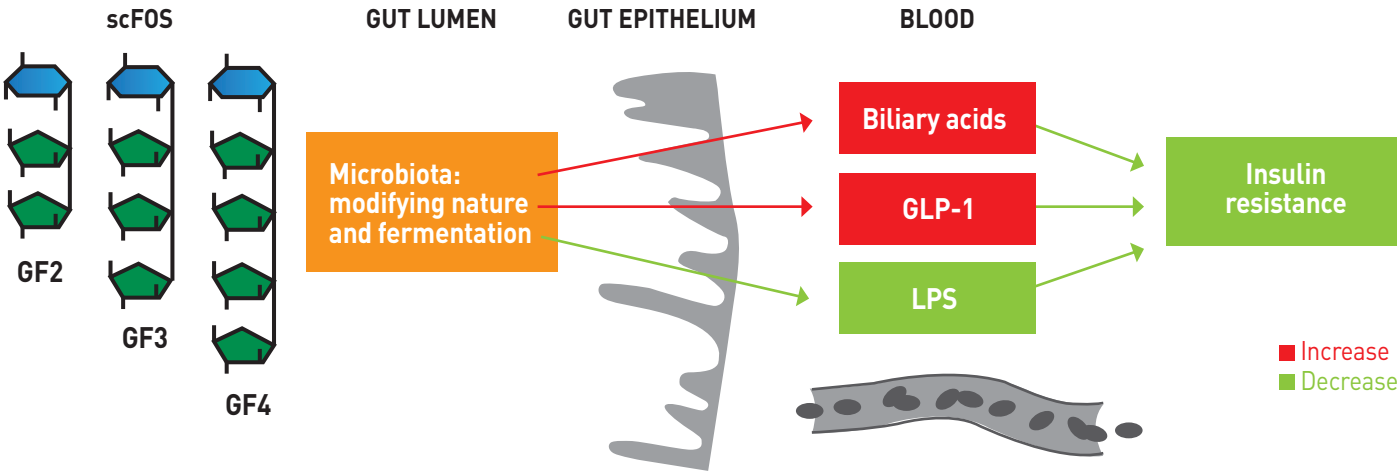

Figure 7. Possible mechanisms that may explain the modulation of insulin sensitivity by PROFEED®. This scheme is based on the knowledge obtained in horses, rodents and dogs.

PROFEED® AND IMMUNITY

PROFEED® modulates the immune response in several species.

PROFEED® is able to modulate the immune response by interacting with the gut-associated lymphoid tissue (GALT). Indeed, PROFEED® shows an impact on different immune markers. It increases the number of Peyer's patches, the number of CD8+ cells, as well as ileal Immunoglobulins A (IgA) concentrations in mice [34, 35] and dogs [16]. In piglets and calves, ingesting scFOS increases the secretive IgA and cell proliferation in Peyer's patches and mesenteric lymph nodes [36; 38].

There is evidence suggesting PROFEED® also modulates the systemic immune response. In dogs and pigs, a supplementation with scFOS during gestation and lactation changes the immune quality of colostrum, probably related to a migration of B-cells from GALT to mammary gland by homing. Besides this effect, the immune responses of puppies and piglets receiving milk from scFOS-supplemented females are stimulated. Indeed, higher serum concentrations of specific IgM for puppies and IgA for piglets are observed when they are submitted to a vaccine challenge [37; 38].

A supplementation with 30 g PROFEED®/day modulates systemic immune parameters.

Similarly to what is observed in other species, PROFEED® modifies immune parameters in horses. Eight horses fed with basal diets supplemented with 30 g maltodextrine or scFOS/day received an intramuscular vaccination against EHV1-EHV4 after a 22-day supplementation. Immune parameters were measured in serum, the digestive contents, and saliva. The vaccine response was not altered by the PROFEED® supplementation, but total serum IgA and serum basophils were increased throughout the experiment, suggesting a systemic effect of scFOS on immunity (Figure 8; [39]).

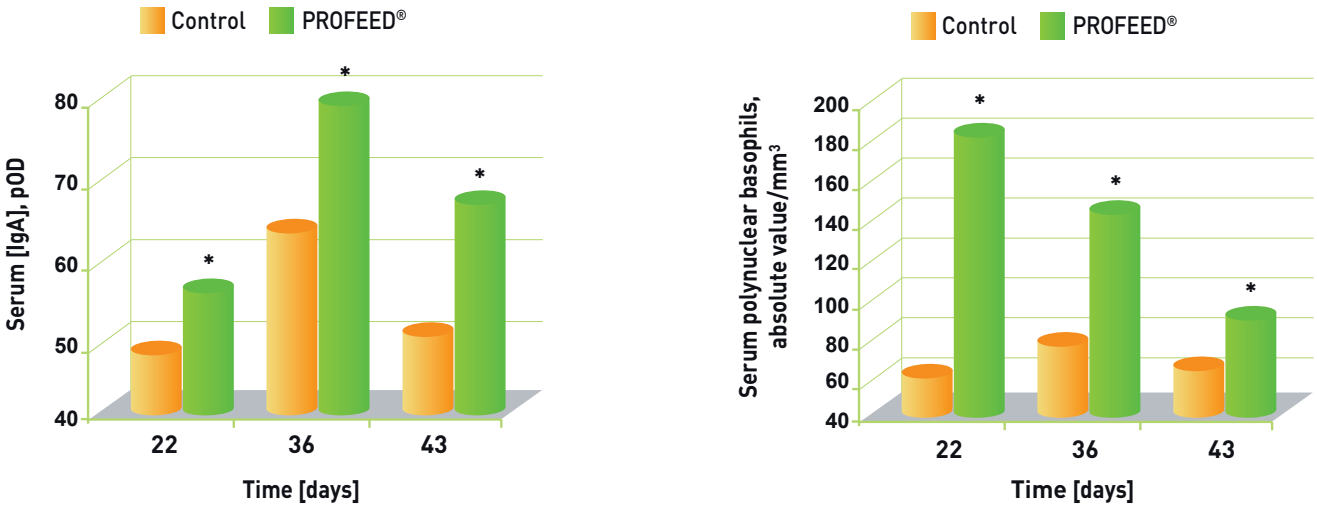

Figure 8. A supplementation with 30 g PROFEED®/day increases serum IgA levels and basophil levels (*p < 0.01 between control and scFOS all the time) [39].

CONCLUSION

A supplementation with 30 to 45 g PROFEED®/day in horse diets:

- balances and stabilises the microbiota, especially in high starch diets,
- limits D-Lactate accumulation in colon and increases acetate and butyrate production,
- reduces digestive disorders,
- increases insulin sensitivity in obese horses,
- modulates the systemic immune responses.

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