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Changes of the hindgut microbiota due to high-starch diet can be associated with behavioral stress response in horses

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HIGHLIGHTS

- Alimentary stress induced increases of colonic anaerobic bacteria concentrations.
- Vigilance was correlated with cecal and colonic amylolytic bacteria concentrations.
- Dietary-induced modulation of the microbiota may affect horse behavior.
- Behavioral cues may be used as non-invasive indicators of alimentary stress.
- Behavioral cues might prove useful to prevent intestinal pain of horses on farms.

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ABSTRACT

The digestive system of horses is adapted to a high-fiber diet consumed in small amounts over a long time. However, during training, high-starch and low-fiber diets are usually fed which may induce hindgut microbial disturbances and intestinal pain. These diets can be described as alimentary stress. The aim of the present study was to investigate to what extent changes in behavior are associated with alimentary stress and microbial composition changes of the cecal or colonic ecosystem. Six fistulated horses were used. The alimentary stress was a modification of diet from a high-fiber diet (100% hay) to a progressive low-fiber and high-starch diet (from 90% hay and 10% barley to 57% hay and 43% barley in 5 days). Cecal and colonic total anaerobic, cellulolytic, amylolytic and lactate-utilizing bacteria were enumerated three times (twice on high-fiber diet and once on 57% hay and 43% barley diet). The behavior of horses was assessed from continuous video recording over an 18-h time period. In addition two personality traits were measured: neophobia (assessed from the reaction to the presence of a novel object placed near a feeder in a test arena) and sociability (assessed from the reaction to an unfamiliar horse in a stall). Video recordings were analyzed by scan sampling every 10 min using the following behavioral categories: lying, resting, feeding and being vigilant. In addition, we recorded time spent feeding and time spent in vigilance during the neophobia test, and time spent in vigilance and time spent in interactions with the unfamiliar horse during the sociability test. The alimentary stress induced significant increases of colonic total anaerobic bacteria, lactate-utilizing bacteria and amylolytic bacteria concentrations. When horses were fed the 57% hay–43% barley diet, time spent in vigilance tended to be positively correlated with cecal and colonic amylolytic bacteria concentrations during the sociability test and with cecal lactate-utilizing and colonic amylolytic bacteria concentrations during the neophobia test. These correlations suggested that dietary-induced modulation of the microbiota may affect horse behavior and that behavioral cues may be used as non-invasive indicators of alimentary stress. It might prove useful to prevent intestinal pain of horses on farms.

1. Introduction

The horses' natural diet is mainly composed of grass, which is typically high in fiber and calorically dilute. Accordingly, the horse digestive system is adapted to make benefits from high-fiber diet consumed in small amounts over a long time [1]. However, the domestication and the use of horses as athletes have led managers to feed them large meals of low-fiber and usually high-starch diets in order to provide more energy. Epidemiological studies reported that the ingestion of a large amount of concentrate or cereals, and therefore starch, induced intestinal pain and strongly increased the risk of colic [2–4]. Moreover several studies showed that high intake of starch created disturbances in the equine large intestine ecosystem, not only in terms of its composition but also

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its activity [5–7]. Therefore a modification of diet from a high-fiber diet to a high-starch and low-fiber diet can be described as an alimentary stress.

In a number of species, behavioral cues are used to assess stressful situations as in novel or sudden situations [8–10]. For instance, alertness postures are considered to be a passive stress response in mammals [11,12]. Moreover horses fed a diet low in fiber and high in grain have a greater risk of developing stereotypic behaviors such as cribbing, circling the stall and weaving [13]. They also spent less time eating and more time standing (resting or being vigilant) than horses fed a diet high in fiber [14]. Hence these behavioral cues may be useful also to assess intestinal pain before diseases, like colic, develop.

Few studies, mostly in laboratory animals, have examined whether shifts in bacterial diversity due to dietary manipulation could be correlated with changes in behavior. For instance, a reduced anxiety-like behavior was observed in a spatial discrimination task in mice fed with a diet inducing a higher colonic bacterial diversity [15]. In addition, anxiety and aggression were increased following raised concentrations of fermentation end products, such as lactic acid and volatile fatty acids in the caecum of rats [16]. In humans, commensal microorganisms within the gut seem to play a role in determining stress reactivity especially through the hypothalamic–pituitary–adrenal axis [for a review 17]. Because intestinal microbiota can interact with the nervous system [18], modification of the microbiota may directly alter behavior. The aim of the present study was to investigate to what extent behavioral changes are associated with changes of the microbiota through an alimentary stress in horses. We discuss the possibility of using behavioral cues as a non-invasive technique to assess alimentary stress.

2. Material and methods

The protocol was approved by the Committee on the Ethics of Animal Experiments of Grand Campus Dijon (registration number: 105; April 05, 2013).

2.1. Subjects and facilities

Six fistulated geldings were used. The barrel of each fistula (in the cecum and in the right-ventral colon) was 15 cm long and 2.25 cm internal diameter. Surgery procedure used to set fistulas was the same as described by Drogoul et al. [19], and was performed at least five years ago. Hence, the effect of fistula on behavior was assumed to be negligible.

Horses were aged 11–19 years and weighing 394 to 511 kg. They were housed in 3.3 × 4 m stalls which contained an automatic waterer (water provided free choice), a plastic feed bucket for hay, a plastic feed bucket for barley with a trace mineral and NaCl block, and wood shavings (Copeaux Classic, Thierwhol, Retteinmaier, France) as bedding over rubber stall mats. Horses were fed at approximately 08:00 and 17:00; stalls were cleaned and bedding replaced each morning. The horses were released daily (from 12:00 to 17:00) in a small dry paddock (10 × 10 m). Horses were exercised five times a week (except days of digestive collection or behavioral tests) in an automatic walker 1 h per day at 6–7 km/h. Their vaccinations against tetanus and influenza (ProteqFlu TE, Merial) and their de-worming (Equest Oral Gel (moxidectin), Fort Dodge Animal Health) were updated before the start of the experiment.

2.2. Experimental design and diets

The horses were submitted to a longitudinal experiment composed of 10 weeks and separated in 3 periods, each associated with a diet. During period 1 (3 weeks), horses were fed a high-fiber diet composed of 100% of hay (H diet; 2.2 kg of Dry Matter (DM)/day/100 kg of Body Weight (BW)). Then, during the period of transition (5 days), they were submitted to a modification of diet from the H diet to a progressive low-fiber and high-starch diet (from 90% hay and 10% barley to 60% hay and 40% barley in 4 days). During period 2 (3 weeks), horses were fed a diet composed of 57% of hay and 43% of barley (HB diet; 1.4 kg of DM/day/100 kg of BW with 0.8 kg of DM/day/100 kg of BW hay). During period 3 (3 weeks), horses were fed an H diet without transition. All the diets were formulated to be iso-energetics and to meet 100% of the energy requirements for horse subjected to a very light work. Hay was offered in equal proportion whereas barley was given as 2/3rd in the morning and 1/3rd in the evening (providing 251 g and 126 g starch/meal/100 kg of BW respectively). Every period, the body weight of each horse was assessed visually through a body condition score and horses were weighed. Timing of the experimental procedures is described in Fig. 1.

2.3. Sampling collection

Blood samples from the jugular vein (5 ml, anticoagulant: EDTA (ethylenediaminetetraacetic acid)) were collected 4 h after the morning meal, the second week of each period (Fig.1).

Cecal and colonic contents were collected 4 h after the morning meal the third week of each period (Fig.1) and sampled in a container, filled to the maximum capacity to avoid the presence of oxygen, for microbiological analyses performed directly after the sample collection.

2.4. Bacteria functional group analysis

The total anaerobic bacteria and the cellulolytic, amylolytic and lactate-utilizing bacteria were enumerated in each cecal and colonic sample using conventional anaerobic culture techniques. All the samples were diluted in a mineral solution under continuous flow of CO_2 to obtain strict anaerobic conditions [20]. The total anaerobic bacteria and the lactate utilizing bacteria were respectively inoculated on a non-selective medium [21,22] and on a selective medium containing lactate [22,23]. Both were cultured anaerobically in roll-tubes [24] and counted after 48 h at 38 °C. The amylolytic bacteria were cultured 48 h at 38 °C on plates containing a medium with soluble starch, and then were enumerated after revelation of colonies with a lugol solution. The cellulolytic bacteria were cultured anaerobically in a complex liquid medium containing one filter paper strip as cellulose source [25]. Their concentration was determined after 14 days at 38 °C thanks to the method of the most probable number of Mc Grady [25]. Finally, all the bacteria concentrations were turned into decimal logarithms.

2.5. Blood analysis

Each blood sample was sent to an external private laboratory (Frank Duncombe, France) to analyze the complete blood count (hematocrit, red blood cells, white blood cells, mononuclear cells, granulocytes and lymphocytes).

2.6. Behavioral analysis

In order to examine the reactions specific to the stimulus studied, we attempted to test the horses' reaction in a context as neutral as possible: sociability and novelty tests were thus performed in a barn adjacent to the stall where horses were living. Both tests were realized the same day in the second week of each period (Fig. 1.).

2.7. Sociability test

Two adjacent and empty stalls (3.3 × 4 m) were used: one stall for the tested horse and one stall for the unfamiliar horse. Each focal individual was placed alone into one stall for 10 min (without the unfamiliar horse in the adjacent stall). Then the unfamiliar horse was introduced into the adjacent stall. The behavior of the two horses was then recorded (using a digital Wireless PIR Camera) during 10 min [26], and continuously analyzed using the following behavior categories: resting, being vigilant and interacting with the unfamiliar horse (head and ears oriented towards the unfamiliar horse when the animal's nose was less than