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1 **Hindgut microbes, fermentation and their seasonal variations in Hokkaido native**
2 **horses compared to light horses**

3
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15
16 Running title: Hindgut microbes in native horses

1 **Abstract:** Fecal bacteria and protozoa of Hokkaido native horses and light horses were
2 enumerated to compare seasonal variation in hindgut microbes and fermentation
3 between the two breeds. Fecal samples were collected in winter and summer from eight
4 horses (4 for each breed) that had been reared together under the same conditions after
5 birth (on woodland pasture in winter and on grassland pasture for the rest of the year).
6 Total fecal bacteria counts for both breeds showed temporal variation, with the highest
7 levels occurring in summer ($P<0.05$). For both breeds, Gram negative rods were the
8 major constituents (58-69%) and showed higher counts in winter ($P<0.05$) than in
9 summer. Total protozoa counts in both breeds were lower in winter than in summer
10 ($P<0.05$). The proportion of large cellulolytic protozoa such as *Cochliatoxum*
11 *periachtum* was increased ($P<0.05$) in winter, and this tended to be more pronounced in
12 native horses. Although total volatile fatty acids (VFA) in feces were lower in winter
13 ($P<0.05$), the reduction was smaller in native horses ($P<0.05$). Fecal VFA pattern
14 showed a shift toward more acetate and less propionate production in winter regardless
15 of the horse breed. Evaluation of digestive tract organs in 12 animals showed that the
16 relative weight of the colon in body weight or total digestive tract weight is larger in
17 native horses than in light horses ($P<0.05$). The present results suggest that hindgut
18 microbial adaptation to winter diets occurs to a greater extent in native horses, as partly
19 characterized by advantages in anatomical structure.

20

21 **Key words:** Hokkaido native horse; hindgut microbes; seasonal change;
22 cellulolytic protozoa; colon

23

24

1 **Introduction**

2

3 Microbes in the large intestine of horses are responsible for fiber digestion. In
4 addition, these symbiotic microbiota, consisting of bacteria, protozoa and fungi,
5 produce volatile fatty acids (VFA) as a means of supplying energy to the host animal
6 (Stewart 1996). Population sizes of specific microbial groups vary in response to
7 differences in feed type, and intestinal digestion changes to reflect feed type (Goodson
8 et al. 1988). However, the factors influencing microbial populations and fermentation in
9 the equine hindgut have not been clearly described. Feed type and intake level alter the
10 retention time of digesta in horses (Dorogoul et al. 2000a), consequently affecting
11 hindgut microbiota composition. In addition, the establishment of microbiota could
12 depend on the hindgut capacity of horses as a reflection of body size or even horse
13 breed.

14 Comparative studies on digestion among horse breeds, particularly breeds of
15 different body sizes, are quite limited. Although ponies are small-bodied, some have
16 been thought to have higher digestive capability for diets of low quality forage (Kondo
17 2001). Kawai et al. (1998) showed that Hokkaido native horses, one of the seven native
18 pony breeds in Japan, tend to have higher digestibility of neutral detergent fiber (NDF)
19 and acid detergent fiber (ADF) than light horses. Lower body weight reduction in
20 Hokkaido native horses compared to that in light horses on winter woodland pasture
21 was also reported (Shingu et al. 2000). Our preliminary observation suggested that the
22 relative size of the large intestines is greater in native horses than the reported value in
23 Thoroughbred (Stewart 1996). In horses, having a proportionally large sized large
24 intestine is considered to be advantageous for longer retention of feed and microbes,

1 which could in turn promote fiber digestion.

2 We studied the differences in hindgut microbe and fermentation characteristics
3 between Hokkaido native horses and light horses that had been raised together in a same
4 manner since birth. This study may clarify the digestive capability of the native horses
5 from the viewpoint of hindgut microbes and fermentation. We also attempted to discuss
6 hindgut microbes in relation to anatomical characteristics, *i.e.* relative weight of large
7 intestine in horses.

8 Although there is no detailed comparative study, we assumed that fecal microbiota
9 could reflect hindgut microbiota to some extent, since no significant difference in
10 microscopic enumeration was observed between the rectum and colon of horses (Koike
11 et al. 2000). This was also reported by Kern et al. (1974) who investigated microbes of
12 the terminal colon (close to rectum) and cecum of the pony. Similarly, fecal sample was
13 used as an alternative to hindgut sample to study hindgut protozoa in the horse (Ike et
14 al., 1985).

15

16 **Methods**

17

18 *Animals, pastures, samplings and study area*

19 A total of eight horses, consisting of 4 Hokkaido native horses (native horses) and 4
20 light horses (75% Thoroughbred and 25% other light breed; light horses), were included
21 in our study. Average body weights with SD at the beginning of the experiment were
22 355 ± 41 and 575 ± 49 kg for native horses and light horses, respectively. All were born
23 at the experimental livestock farm of the Northern Biosphere Field Science Center of
24 Hokkaido University, Shizunai, Japan ($42^{\circ}25'$ N, $142^{\circ}28'$ E) and raised under the

1 exactly same conditions. The animals were in direct contact with each other for a long
2 period of time, allowing ample chance for microbial transfer to occur. The animals were
3 conventionally reared with grazing on grassland pasture from spring to autumn (from
4 April through November) and on woodland pasture in winter (from December through
5 March). The grassland pasture was primarily occupied by timothy, while the
6 undergrowth of the woodland pasture was bamboo grass (*Sasa nipponica*). On winter
7 woodland pasture, the horses also had access to bark and twigs when the bamboo grass
8 was depleted or covered with snow.

9 The experiment was comprised of three sampling periods: the first sampling in
10 January, 2000 (1st winter), the second in August, 2000 (summer) and the last in January,
11 2001 (2nd winter). During these periods, freshly deposited feces were collected for
12 microbial and fermentation analyses. The samples were taken from the interior of the
13 feces to avoid contamination by soil microorganisms and kept in an icebox until further
14 processing. According to climate data for this area during the experimental period, the
15 average temperature ranged from 20.4°C in August to -4.4°C in February. Snowfall
16 occurred from November until March with largest accumulation of 37 cm in January,
17 2000.

18

19 ***Microbial enumeration and VFA analysis***

20 Fecal samples were homogenized with saline containing 20% chloroform and
21 preserved at room temperature until analysis. Microscopic observation was made to
22 morphologically enumerate fecal bacteria according to Suto's methods (Suto 1973). In
23 brief, homogenized feces (1g) were diluted with saline ($\times 10^{2-3}$), a part of which was
24 fixed on a glass plate after mixing with agar solution. Then following Gram staining,

1 counts with morphological classification for selected 16 fields per sample were made
2 under a light microscope (BX51, Olympus, Tokyo, Japan) fitted with a micrometer (\times
3 1,000). Bacteria were classified into four categories: rods and cocci being either Gram
4 positive or Gram negative. For analysis of protozoa, feces (1g) were gently mixed with
5 MFS (Methylgreen Formalin Saline) solution (4ml) and enumerated ($\times 400$) and
6 counted ($\times 100$) under a microscope. The enumerative counting was performed twice
7 per sample. These procedures and protozoa identification were carried out according to
8 Ogimoto and Imai (1981).

9 VFA analysis and pH determination was carried out with the supernatant of feces
10 that were homogenized in distilled water and centrifuged ($10,000 \times g$). VFA analysis
11 was made by gas chromatography (GC14B, Shimazu, Kyoto, Japan) with total VFA
12 being expressed in terms of fecal dry matter (Suto 1973). All the chemicals used were of
13 reagent grade (Wako, Osaka, Japan).

14

15 *Collection of anatomical data*

16 Weights of digestive organs were determined on a total of 12 horses, 6 native horses
17 (191 ± 28 kg in body weight; 1-2 y of age) and 6 light horses (100% Thoroughbred; 562
18 ± 30 kg; 14-15 y), which were killed at a local slaughterhouse. All of these horses had
19 been raised mainly on a timothy hay/wheat bran diet in the same area of Shizunai.
20 Alimentary tracts for all horses were dissected, washed, weighed and separated into
21 cecum, colon and others. The separated cecum and colon were also weighed. Since fiber
22 digestion occurs mainly in the ventral and dorsal colon (Hintz *et al.* 1971; Drogoul *et al.*
23 2000b), the distal small colon was excluded from the “colon” in this study.

24

1 *Statistical analyses*

2 Data were expressed as means with standard errors of four replications (microbial
3 and fermentation data) or six replications (anatomical organ data) within each breed.
4 The microbial and fermentation data were analyzed using a 3 x 2 factorial arrangement
5 using the GLM procedure of SAS with the factors of season (1st winter vs. summer vs.
6 2nd winter), breed (native horse vs. light horse), and their interaction. When a significant
7 difference among the seasons was detected, multiple comparisons between seasons were
8 made using the least significant difference (LSD) test. The anatomical data were
9 compared between the breeds (native horse vs. light horse) by Student's t-test.

10

11 **Results**

12

13 *Bacteria*

14 Total fecal bacteria counts and morphological classifications of bacteria are shown
15 in Table 1. Total counts varied with season, with highest counts in summer ($p < 0.05$).
16 There were no breed-specific changes in total bacterial counts.

17 Gram negative rods formed the majority of the bacteria (ranging from 58.1 to
18 69.1%) followed by Gram negative cocci (26.3 to 37.0%), Gram positive rods (1.5 to
19 6.4%) and Gram positive cocci (1.2 to 3.5%). There were consistent seasonal changes in
20 the proportions of Gram negative cocci and rods; higher proportions of Gram negative
21 rods were noted in both breeds in the winters ($p < 0.05$), while lower proportions of
22 Gram negative cocci were recorded in the corresponding periods. There were no
23 significant differences in the proportions of enumerated bacterial groups between native
24 horses and light horses.

1

2 ***Protozoa***

3 Seasonal changes in total fecal protozoa counts and generic composition in native
4 horses and light horses are presented in Table 2. There was an apparent seasonal shift in
5 total protozoa counts in native horses, with an increase from the first winter to summer
6 ($p < 0.05$) and a reduction in the second winter ($p < 0.05$). For light horses, an increase in
7 summer was not significant, while a decrease in the second winter was apparent
8 ($p < 0.05$).

9 *Bundleia postciliata* was the most common protozoa species (39.2-54.7%) in the
10 hindgut of the horses examined here, irrespective of breed, followed by *Didemnis*
11 *quadrata* (5.8-17.2%) and *Ditoxum funinucleum* (2.0-15.8%). These major species
12 showed no significant changes with season, but showed great variation among
13 individual animals. Meanwhile, a fibrolytic, large-sized protozoon *Cochliatoxum*
14 *periachtum* showed a typical seasonal change. *C. periachtum* was present in higher
15 proportions in winters compared to the summer ($P < 0.05$). This species was undetectable
16 in light horses in summer, and as a result, the abundances of this species were
17 significantly higher in native horses ($P < 0.05$). Active engulfment of plant fragments by
18 this large protozoon was frequently observed, especially in native horses (Fig. 1).

19

20 ***Fermentation***

21 Table 3 shows fecal pH, total VFA concentration and molar proportions of VFA.
22 Fecal pH decreased in summer and increased in winters, corresponding to the opposite
23 changes observed for total VFA concentrations ($P < 0.05$). There were differences in the
24 total VFA concentrations between the horse breeds, with higher values observed in

1 native horses ($P < 0.05$). Fermentation patterns showing more acetate and less propionate
2 were observed in winters, irrespective of horse breed.

3

4 *Anatomical parameters*

5 Body weight and anatomical data for the digestive tract are given in Table 4.
6 Absolute weights of the whole body, total digestive tract, cecum and colon were lower
7 in native horses ($P < 0.001$). When tissue weights were expressed as percentages per
8 body weight and per weight of total digestive tracts in order to normalize for the
9 different body sizes for the two breeds, the relative colon weight was higher in native
10 horses than in light horses ($P < 0.05$). Meanwhile, the relative weight of cecum did not
11 differ significantly between the two breeds. The relative weight of the colon plus cecum
12 was higher in native horses compared to light horses ($P < 0.01$).

13

14 **Discussion**

15

16 Hokkaido native horses have been considered to be able to digest lower quality
17 forage compared to light breeds such as Thoroughbreds (Kondo 2001). Although their
18 behavioral advantage in grazing on woodland pasture had been reported (Shingu *et al.*
19 2000), evaluations from a nutritional viewpoint had not been undertaken.

20 The superior digestive ability in native horses was suggested to be due to anatomical
21 advantages such as a relatively enlarged colon (Table 4). This allows plant fibers to be
22 held for longer periods of time in the hindgut, thus promoting fiber digestion. These
23 anatomical advantages, also promote longer residence time of hindgut microbes, which
24 could facilitate the establishment of fibrolytic microbes in the hindgut since they require

1 longer doubling times than other microbes (Van Soest 1994). Although no reports on the
2 differences of digesta passage between native horses and light horses are available, the
3 above anatomical differences given in terms of body weight could produce differing
4 retention times of digesta and microbes since animals are generally fed based on body
5 weight. The colon is considered to be the main site of fibrolysis in horses (Hintz *et al.*
6 1971; Drogoul *et al.* 2000b). The present results show a larger relative weight for the
7 colon in native horses, suggesting that they may have superior fiber digestion.

8 Since bacterial enumeration was performed, based on Gram staining and
9 morphology, it is not possible to make inferences on taxonomy. However, there was a
10 clear shift in the proportion of Gram negative rods, showing significant increases in the
11 winter seasons (Table 1). Koike *et al.* (2000) found that Hokkaido native horses have a
12 bacterial adaptation to low-quality roughage diets on winter woodland pasture in their
13 increased levels of *Fibrobacter succinogenes*, one of the most potent fibrolytic species
14 present in the equine hindgut. They also observed an increase in Gram negative rods in
15 winter (Koike *et al.* 2000). Since *F. succinogenes* is categorized with the Gram negative
16 rods, the increased proportion of this group in winters of the present study (Table 1)
17 may be partly explained by the increase of *F. succinogenes* in winter up to 1.0-4.4% of
18 total bacteria (Koike *et al.* 2000).

19 Although Jullian *et al.* (1999) pointed out the significance of *Ruminococcus*
20 *flavefaciens* in fiber digestion of horses, we have already confirmed that this species is
21 minor in Hokkaido native horses (Koike *et al.* 2000). Instead of *R. flavefaciens*, novel
22 groups of *F. succinogenes* in the equine hindgut (Lin & Stahl 1995) should be explored
23 in our case. Even though no direct comparisons were made for cellulolytic bacterial
24 counts between native horses and light horses, reported values show that Shetland

1 ponies, similar to our native horses in body size, have higher numbers of cellulolytic
2 bacteria than light horses (10^7 vs. 10^6 /g) (Kern et al. 1973, 1974; Mackie & Wilkins
3 1988; Moore & Dehority 1993).

4 Total bacterial counts showed seasonal variations, with higher levels in summer than
5 in the winter seasons, as was also noted in the preceding report (Koike *et al.* 2000) in
6 which the winter feed supply was thought to be lacking in both quantity and quality.
7 These situations could limit the growth of a wide variety of bacterial species. This could
8 result in the selection of fibrolytic species, possibly accompanying a reduction in the
9 passage rate of digesta.

10 Hindgut fauna, mainly comprised of small-sized protozoa, is considered to play a
11 minor role in feed digestion in the equine hindgut, as indicated in a defaunation study
12 (Moore & Dehority 1993). However, the present results on seasonal protozoal variation
13 suggest that large-sized fibrolytic protozoa may support some extent of forage digestion,
14 especially in native horses (Table 2). In fact, fibrolytic *C. periachtum* (Ogimoto & Imai
15 1981) increased its relative proportion in winter in native horses (Table 2). Abundance
16 of this species in the hindgut was able to fluctuate in native horses so as to adapt to the
17 more fibrous diet in winter. This hypothesis is partly supported by the direct observation
18 of plant fragments being actively ingested by this protozoon (Fig.1). This is not the case
19 for light horses, probably due to the lower relative capacity of the hindgut on total
20 digestive tract weight basis (Table 4), which does not allow a longer residence of
21 large-sized and slow-growing fibrolytic protozoa (Van Soest 1994). In fact, *C.*
22 *periachtum* is found at lower levels in light horses (even becoming undetectable level in
23 summer) (Table 2).

24 Lower fecal VFA levels with proportionally more acetate and less propionate in

1 winter (Table 3) explain the seasonal microbial selection due to diet changes from
2 pasture grass (summer) to bamboo, bark and others (winter) discussed above, as
3 fibrolytic bacteria and protozoa are acetate producers (Stewart 1996). Higher VFA levels,
4 especially higher acetate levels, in native horses even during winter (Table 3) may
5 suggest that microbial adaptation to fibrous diet is greater in native horses than in light
6 horses. A similar shift of fermentation pattern is reported in the rumen of wild sika deer
7 ingesting highly fibrous foods such as bark in snowy winter (Ichimura *et al.* 2004).
8 Floral shift of horse hindgut in the present study (more Gram negative rod in winter,
9 Table 1) was opposite to that of deer rumen (less Gram negative rod but more Gram
10 negative cocci in winter, Ichimura *et al.* 2004), implying the difference in floral
11 members between these two animal species. Thus, microbial adaptation could be
12 microbial community shift to maximize feed digestion in a certain situation.

13 The present study indicates the alteration of hindgut microbiota with season (dietary
14 condition) and horse breed. In particular, shifts in micro-fauna as well as micro-flora,
15 corresponding to seasonal changes, are more profound in Hokkaido native horses,
16 which have larger relative colon volumes than light horses. This anatomical character in
17 native horses could be advantageous to fibrolytic bacteria and protozoa, which
18 contribute to the digestion of low quality roughage while producing more acetate.
19 According to digestion trials using a double indicator technique, NDF digestibility of
20 bamboo grass during grazing was higher, but not significantly so, in native horses than
21 in light horses (38.6 vs. 32.1%) (Shingu *et al.* 2000).

22 Adaptation of gut microbiota in wildlife to seasonal or dietary conditions has been
23 described for the ruminants deer (Orpin *et al.* 1985; Dehority & Demarais 1999;
24 Ichimura *et al.* 2004) and sheep (Hobson *et al.* 1975). Our results suggest that this

1 adaptation is also present in the equine hindgut, the extent of which would be greater in
2 Hokkaido native horses than in light horses. Further nutritional implications of this
3 phenomenon need to be more closely examined in digestion trials involving the
4 determinations of microbes, fermentation and digesta passage.

5 The differences in anatomical data between native horses and light horses should be
6 carefully discussed, considering difference of age (1.5 vs. 14.5 years of age). In fact, the
7 slaughtered native horses were not fully matured. They may have higher relative weight
8 of digestive tract on body weight basis when compared with matured animals, since
9 bone and digestive tract development comes first, followed by muscle development and
10 fat deposition (Gill & Oldham 1993). Meanwhile, Cunha (1991) describes that
11 development of large intestine is completed by 15-24 months old that is the age of the
12 slaughtered native horses in the present study. Based on these, it might be true that at
13 least the relative weight of hindgut on total tract weight basis is a valid index to mention
14 anatomical difference between native horses and light horses even in different maturity.

15

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17

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21

22 **References**

23

24 Cunha T.J. (1991) The digestive tract. In: *Horse feeding and nutrition*. (ed T.J.Cunha),

- 1 pp.28-38.Academic Press, San Diego.
- 2
- 3 Dehority B.A., Demarais S. & Osborn D.A. (1999) Rumen ciliates of white-tailed deer,
4 axis deer, sika deer, fallow deer from Texas. *Journal of Eukaryotic Microbiology*
5 **46**:125-131.
- 6
- 7 Drogoul C., Poncet C. & Tisserand J.L. (2000a) Feeding ground and pelleted hay rather
8 than chopped hay to ponies. 1. Consequences for in vivo digestibility and rate of
9 passage of digesta. *Animal Feed Science and Technology* **87**:117-130.
- 10
- 11 Drogoul C., Poncet C. & Tisserand J.L. (2000b) Feeding ground and pelleted hay rather
12 than chopped hay to ponies. 2. Consequences on fiber degradation in the cecum and the
13 colon. *Animal Feed Science and Technology* **87**:131-145.
- 14
- 15 Gill M. & Oldham J.D. (1993) Growth. In: *Quantitative aspects of ruminant digestion*
16 *and metabolism*. (ed J.M.Forbes & J.France), pp.383-403. CAB International,
17 Wallingford.
- 18
- 19 Goodson J., Tyznik E.J., Cline J.H. & Dehority B.A. (1988) Effect of an abrupt diet
20 change from hay to concentrate on microbial numbers and physical environment in the
21 cecum of the pony. *Applied and Environmental Microbiology* **54**:1946-1950.
- 22
- 23 Hintz H.F., Hogue D.E., Walker Jr.E.F., Lowe J.E. & Schryver H.F. (1971) Apparent
24 digestion in various segments of the digestive tract of ponies fed diets with varying

- 1 roughage-grain ratios. *Journal of Animal Science* **32**:245-248.
- 2
- 3 Hobson P.N., Mann S.O. & Summers R. (1975) Rumen micro-organisms in red deer,
4 hill sheep and reindeer in the Scottish highlands. *Proceedings of Royal Society of*
5 *Edinburgh (B)* **75**:171-180.
- 6
- 7 Ichimura Y., Yamano H., Takano T., Koike S., Kobayashi Y., Tanaka K., Ozaki N.,
8 Suzuki M., Okada H. & Yamanaka M. (2004) Rumen microbes and fermentation of
9 wild sika deer on the Shiretoko peninsula of Hokkaido island, Japan. *Ecological*
10 *Research* **19**:389-395.
- 11
- 12 Ike K., Imai S. & Ishii T. (1985) Establishment of intestinal ciliates in new-born horses.
13 *Japanese Journal of Veterinary Science* **47**:39-43.
- 14
- 15 Julliand V., DeVaux A., Millet L. & Fonty G. (1999) Identification of *Ruminococcus*
16 *flavefaciens* as the predominant cellulolytic bacterial species of the equine cecum.
17 *Applied and Environmental Microbiology* **65**:3738-3741.
- 18
- 19 Kawai M., Shingu Y., Inaba H., Kondo S., Hata H. & Okubo M. (1998) Comparison in
20 intake and digestibility of *Sasa nipponica* between Hokkaido native horses and light
21 horses grazed on woodland pasture. *Proceedings of 10th Japanese Society of Equine*
22 *Science*, p 24.
- 23
- 24 Kern D.L., Slyter L.L., Weaver J.M., Leffel E.C. & Samuelson G. (1973) Pony cecum vs

- 1 steer rumen: the effect of oats and hay on the microbial ecosystem. *Journal of Animal*
2 *Science* **37**:463-469.
- 3
- 4 Kern D.L., Slyter L.L., Leffel E.C., Weaver J.M. & Oltjen RR. (1974) Ponies vs steers:
5 microbial and chemical characteristics of intestinal ingesta. *Journal of Animal Science*
6 **38**:559-564.
- 7
- 8 Koike S., Shingu Y., Inaba H., Kawai M., Kobayashi Y., Hata H., Tanaka K. & Okubo
9 M. (2000) Fecal bacteria in Hokkaido native horses as characterized by microscopic
10 enumeration and competitive polymerase chain reaction assays. *Journal of Equine*
11 *Science* **11**:45-50.
- 12
- 13 Kondo S. (2001) *Zoology of horses*. University of Tokyo Press, Tokyo.
- 14
- 15 Lin C. & Stahl D.A. (1995) Taxon-specific probes for the cellulolytic genus *Fibrobacter*
16 reveal abundant and novel equine-associated populations. *Applied and Environmental*
17 *Microbiology* **61**:1348-1351.
- 18
- 19 Mackie R.I. & Wilkins C.A. (1988) Enumeration of anaerobic bacterial microflora of
20 the equine gastrointestinal tract. *Applied and Environmental Microbiology*
21 **54**:2155-2160.
- 22
- 23 Moore B.E. & Dehority B.A. (1993) Effects of diet and hindgut defaunation on diet
24 digestibility and microbial concentrations in the cecum and colon of the horse. *Journal*

- 1 *of Animal Science* **71**:3350-3358.
- 2
- 3 Ogimoto K. & Imai S. (1981) In: *Atlas of Rumen Microbiology*. Japan Scientific
4 Societies Press, Tokyo.
- 5
- 6 Orpin C.G., Mathisen S.D., Greenwood Y. & Blix A.S. (1985) Seasonal changes in the
7 ruminal microflora of the high-arctic Svalbard reindeer (*Rangifer tarandus*
8 *platyrhynchus*). *Applied and Environmental Microbiology* **50**:144-151.
- 9
- 10 Shingu Y., Kawai M., Inaba H., Kondo S., Hata H. & Okubo M. (2000) Voluntary intake
11 and behavior of Hokkaido native horses and light half-bred horses in woodland pasture.
12 *Journal of Equine Science* **11**:69-73.
- 13
- 14 Stewart C.S. (1996) Microorganisms in hindgut fermentors. In: *Gastrointestinal*
15 *Microbiology*, Vol.2 (eds R.I.Mackie, B.A.White BA & R.E.Issacson), pp.162-171.
16 Chapman & Hall, New York.
- 17
- 18 Suto T. (1973) Examination of the rumen. In: *The clinical investigation of the bovine*.
19 (eds R.Nakamura, H.Yonemura & T.Suto), pp.11-14. Nosangyoson Bunka Kyokai,
20 Tokyo.
- 21
- 22 Van Soest P.J. (1994) Microbes in the gut. In: *Nutritional ecology of the ruminant*. (ed
23 P.J.Van Soest), pp.253-280.Cornell University Press, Ithaca.
- 24

Table 1 Fecal bacteria and morphological enumeration in Hokkaido native horses and light horses.

Breed	Season	Total bacteria ($\times 10^{10}$ /g)	Gram positive		Gram negative	
			cocci (%)	rods (%)	cocci (%)	rods (%)
Native horses	1st Winter	1.2 a	1.2	1.5	28.2 a	69.1 a
	Summer	9.2 c	1.8	3.1	37.0 b	58.1 b
	2nd Winter	3.8 b	1.8	3.0	31.3 a	63.9 a
Light horses [†]	1st Winter	1.3 a	3.5	6.4	26.3 a	63.8 a
	Summer	14.9 b	1.5	3.4	34.9 b	60.2 b
	2nd Winter	3.2 c	2.9	5.2	27.1 a	64.8 a
Statistics						
	Breed	NS	NS	NS	NS	NS
	Season	**	NS	NS	*	*
	Breed x Season	NS	NS	NS	NS	NS

Each value represents the average from 4 horses.

a, b: Values with different superscripts within the same column for each breed differ significantly ($P < 0.05$).

[†]: Light horses are 75% Thoroughbred and 25% other light horse breeds.

*: $P < 0.05$; **: $P < 0.01$

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Table 2 Fecal protozoa and generic enumeration in Hokkaido native horses and light horses.

Breeds	Season	Total protozoa	<i>Bundleia</i> <i>postciliata</i>	<i>Ditoxum</i> <i>funinucleum</i>	<i>Triadinium</i> <i>caudatum</i>	<i>Tripalmaria</i> <i>dogieri</i>	<i>Didesmis</i> <i>quadrata</i>	<i>Cycloposuhium</i> <i>bipalmatum</i>	<i>Cochliatoxum</i> <i>periachtum</i>	<i>Blepharocorys</i> <i>uncinata</i>
		($\times 10^4$ /g)	(%)							
Native horses	1st Winter	1.2 a	48.5	15.8	2.4 ab	6.5	8.7	6.9	5.9 b	5.3
	Summer	1.9 b	49.7	2.0	5.7 b	4.8	7.8	5.8	1.1 a	23.2
	2nd Winter	1.0 a	39.2	6.6	0.6 a	16.4	10.0	12.2	5.6 b	9.4
Light horses [†]	1st Winter	1.3 ab	54.6	11.5	1.1 a	5.0	17.2	3.2	2.9	4.5
	Summer	1.7 b	45.7	10.3	8.3 b	11.0	5.8	11.3	ND	7.6
	2nd Winter	0.9 a	54.7	10.1	0.1 a	6.5	13.7	8.6	2.1	4.3
Statistics										
	Breed	NS	NS	NS	NS	NS	NS	NS	*	NS
	Season	*	NS	NS	*	NS	NS	NS	*	NS
	Breed x Season	NS	NS	NS	NS	NS	NS	NS	NS	NS

Each value represents the average for 4 horses. ND: not detected.

a, b: Values with different superscripts within the same column for each breed differ significantly ($P < 0.05$).

[†]: Light horses are 75% Thoroughbred and 25% other light horse breeds.

*: $P < 0.05$

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Table 3 Fecal pH and volatile fatty acids in Hokkaido native horses and light horses.

Horse breeds	Seasons	pH	Volatile fatty acids				
			Total (m moles/g DM)	Acetate	Propionate	Butyrate	Others
Native horses	1st Winter	6.79 a	0.09 a	68.2 a	21.5 a	7.0	3.3
	Summer	6.37 b	0.16 b	62.1 b	26.6 b	7.4	3.9
	2nd Winter	6.59 ab	0.10 ab	66.5 a	22.9 a	7.4	3.2
Light horses [†]	1st Winter	6.85 a	0.05 a	69.1 a	20.8 a	6.5	3.6
	Summer	6.35 b	0.15 b	62.5 b	27.6 b	6.9	3.0
	2nd Winter	6.70 a	0.06 a	67.8 a	21.9 a	7.0	3.3
Statistics							
	Breed	NS	*	NS	NS	NS	NS
	Season	*	*	*	*	NS	NS
	Breed x Season	NS	NS	NS	NS	NS	NS

Each value represents the average for 4 horses.

a, b: Values with different superscripts within the same column for each breed differ significantly ($P < 0.05$).

[†]: Light horses are 75% Thoroughbred and 25% other light horse breeds.

*: $P < 0.05$

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Table 4 Comparison of digestive organs between Hokkaido native horses and light horses.

Parameter of digestive tissue	Breed		Difference
	Native horses (n=6)	Light horses [†] (n=6)	
Body weight (kg)	190.8 (34)	561.8 (100)	***
Total digestive tract (kg)	12.8 (40)	31.8 (100)	***
Cecum (kg)	2.0 (47)	4.3 (100)	***
Colon (kg)	5.4 (52)	10.3 (100)	***
% per body weight			
Cecum	1.1 (138)	0.8 (100)	NS
Colon	2.9 (161)	1.8 (100)	*
Cecum plus colon	4.0 (154)	2.6 (100)	*
% per total tract weight			
Cecum	15.6 (116)	13.5 (100)	NS
Colon	42.2 (130)	32.4 (100)	*
Cecum plus colon	57.8 (126)	45.9 (100)	**

Values in parentheses are relative percentages when the weights of light horses are expressed as 100.

[†]: Light horses are 100% Thoroughbred.

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

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3 Fig. 1.

4 *Cochliatoxum periachtum*, a protozoon notably present in feces of

5 Hokkaido native horses, actively ingesting plant fragments

6 (light microscopy, magnification: $\times 400$; bar scale: $100\mu\text{m}$).

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