Irish Veterinary Journal

RESEARCH

Open Access

Dietary supplementation of new-born foals with free nucleotides positively affects neonatal diarrhoea management

Livio Penazzi[1*], Eleonora Pagliara[1], Tiziana Nervo[1], Ugo Ala[1], Andrea Bertuglia[1], Giovanna Romano[2], Jasmine Hattab[3], Pietro Giorgio Tiscar[3], Stefania Bergagna[4,4], Giulia Pagliasso[4], Sara Antoniazzi[5], Laura Cavallarin[5], Emanuela Valle[1] and Liviana Prola[1]

Abstract

Foals commonly experience diarrhoea in the first weeks of life. Although this condition is rarely life-threatening, it can have significant health consequences. This study investigated whether new-born foals can benefit from a dietary supplement of nucleotides, as already demonstrated in other species. Dietary nucleotides have positive effects on rapidly proliferating tissues and are considered "semi-essential nutrients" since cells have only a limited capacity to synthesize these compounds. The aim of this study was to investigate whether providing foals with a dietary nucleotide supplementation, in the form of an oral paste, was able to affect diarrhoea incidence, systemic immunity, intestinal microbiota and volatile fatty acid production. Thirty new-born standardbred foals, from 3 different premises within the same area, were equally distributed between two groups: one group received an oral paste containing dietary nucleotides (NUCL group), while the other received a placebo paste (CTRL group). Faecal and blood samples were collected on days 1 and 35 after birth. No statistical differences in cytokines (TNF-α, IFN-γ, IL-6, IL-12) or faecal calprotectin levels were found between the two groups, suggesting that the level of nucleotide supplementation used in this study did not have significant effects on the systemic immune system and on the levels of faecal calprotectin. However, the NUCL group showed a lower relative frequency of number of days with diarrhoea (6.12% vs 13.33%; $p < 0.001$) and greater weight gain compared with the CTRL group (50.3 ± 5.65 kg vs 44.0 ± 8.65 kg; $p < 0.05$). Total volatile fatty acids, branched volatile fatty acids, acetic acid, propionic acid, butyric acid, succinic acid and iso-butyric acids in faecal samples were all higher in the NUCL group compared with the CTRL group. This outcome may explain an earlier establishment of a gut microbiota in the foals of the NUCL group that was closer to that typical of an adult horse, characterised by predominant fibrolytic populations. Volatile fatty acid production (especially butyric acid) has also been shown to correlate with the intestinal well-being of the horse, supporting the use of dietary nucleotide supplements for improved health and well-being in new-born foals. Although we noted no clear differences in the faecal microbial communities between the two groups, dietary nucleotide supplementation did appear to have a positive clinical outcome, reducing the number of days of diarrhoea and increasing the levels of volatile fatty acids.

Keywords Nutrition, Microbiota, Volatile fatty acids, Equine, Neonatology, Supplement, Nutrient, Nucleotide

*Correspondence:
Livio Penazzi
livio.penazzi@unito.it
Full list of author information is available at the end of the article

Part one of this paper appeared in Veterinary Ireland Journal, Volume 16, Number 8. Part two begins on the next page:

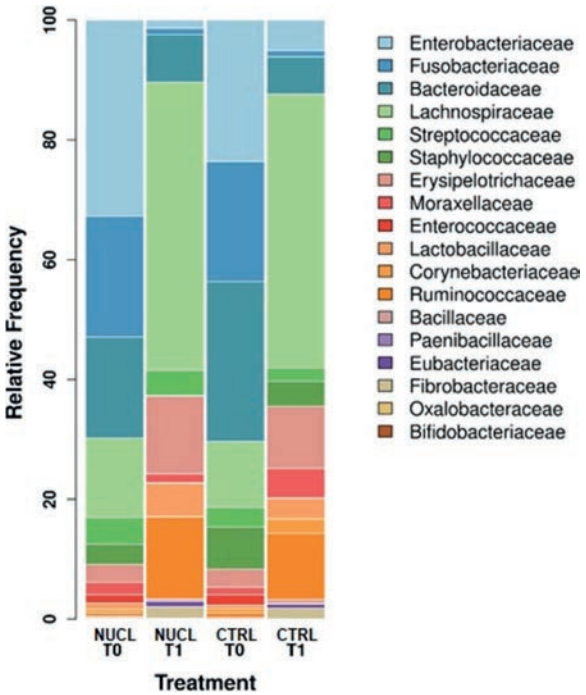

Fig. 7 Average faecal bacterial microbiota of the foals divided according to time of collection (T0 vs T1) and treatment group (NUCL vs CTRL). Bacterial microbiota structures at the family level on day 1 (T0) and day 35 (T1) of birth. The two experimental groups share the same predominant families, but a change in the relative abundance of each family was observed between the two clusters

Previous studies in animals [77, 79] and human neonates [80, 81] showed increased weight gain when dietary nucleotides were added to the diet. Similarly, the foals receiving the nucleotide supplementation in the present study showed greater weight gain compared with those in the control group. As hypothesized by Jang et al. [79], this effect could be a consequence of reduced intestinal inflammation and improved intestinal villi structure and energy digestibility. Nonetheless, the observed effects on the microbiota could also have played a role in their better growth performance. The importance of the microbiota in foal development is crucial as it can be affected by different dynamics, including exposure to transient, non-colonizing organisms via the mare and the environment [54], adaptation to rapid and continuous changes in the diet [21], and periods of coprophagic behaviour [8]. Marked changes in the microbiota can have a direct effect on foal health throughout the first 2 months of life, after which the bacterial populations tend to stabilize [1, 19–21]. The results of PCoA in our study revealed a tighter clustering of the relative abundances of faecal bacterial families in the supplemented foals at T1, indicating a better stabilization of their microbiota compared with the control group. In accordance with De La Torre et al. [10], who found increased levels of *Enterobacteriaceae* in diarrheic foals at seven days of age with respect to healthy foals, the CTRL group at T1 showed a higher relative abundance of this family compared with the NUCL group. Furthermore, Schoster et al. [1] reported that foals exhibiting a higher prevalence of diarrhoea had lower abundances of *Lachnospiraceae* and *Ruminococcaceae*, as also observed in the CTRL group of our study compared with the supplemented foals at T1.

Nucleotides are reported to enhance butyrate-producing bacteria and genera, such as *Ruminococcus* and *Intestinomonas*, in infants [82]. When considering composition changes (in both groups) between T0 and T1, only the Ruminococcaceae family showed an increase in relative abundance, while Lachnospiraceae was the only fibre-degrading bacterial family to exhibit an increase in relative abundance at T1. This family is regarded a plant polysaccharide-degrader and producer of butyrate [83]. As regards the low abundances of the other fibre-degrading bacteria investigated (Fig. 8, violin plot), this might be explained by the fact that other fibre-degrading organisms (such as fungi and yeasts) play a major role in this pathway in horses. In fact, the degradation of plant fibre is a complex process relying on the combined actions of fungi, bacteria and archaea, and recent studies have highlighted the prominent role of yeasts and fungi in the fibrolytic process [84].

Overall, the faecal bacterial communities found in the present study were in line with the literature, which reports the intestinal microbiota of new-borns to be "non-specific", only converging over time towards an adult-like one [24]. The differences between the NUCL and CTRL groups were not very remarkable, but this may be due to the much greater differences that characterize the microbiota of new-born versus 35-day-old foals. More precisely, the larger time-dependent changes in the microbiota could have somehow masked smaller differences in the composition of the microbial communities. There is also some evidence that dietary nucleotides may promote the early determination of gastrointestinal bacterial communities, supporting the stabilization of microbiota, as observed in the present study via PCoA clustering. Thus, the higher level of clustering observed in the NUCL group could depend on the maturation of the gut bacterial communities fostered by nucleotide treatment. Furthermore, administration of different quantities of nucleotides was shown to produce different degrees of variation in the bacterial microbiota and metabolites within the host [85]. Further studies would be useful to investigate the correlation between the quantity of nucleotide ingestion and the extent of changes in the microbiota and immune function, as nucleotides administration seem to affect both of them [24].

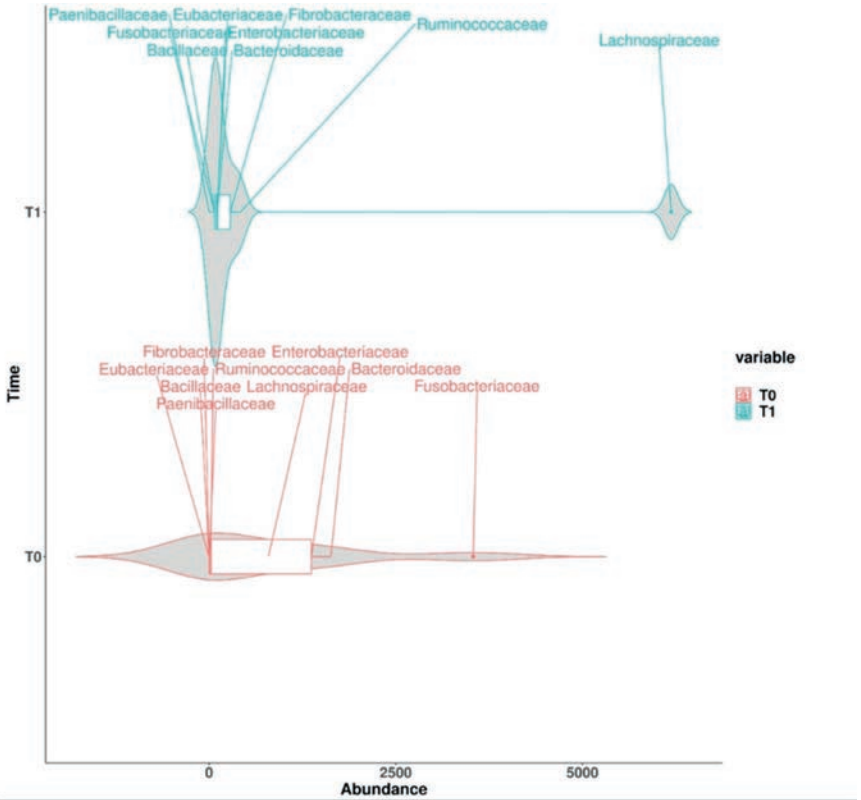

Fig. 8 Violin plot showing the shift in the relative faecal abundances of bacterial families involved in digestion and volatile fatty acid production in foals between the day 1 (T0) and day 35 (T1) of birth. At T0 the examined families display a wider distribution pattern and are more abundant compared with at T1, when the abundance of each family was uniformly low, with the exception of Lachnospiraceae

Similarly, it may be worth exploring if early stabilization of the gut flora could have an effect in the long term during the horse life.

In the present work, foals from NUCL and CTRL group were also observed to have different clinical outcomes with respect to diarrhoea presentation. This is particularly relevant considering that both groups came from the same breeding farms and the animals were exposed to identical environmental and management conditions. In addition, significant differences were detected in faecal VFA between the two groups at T1, with higher total VFA in the NUCL group compared with controls. Volatile fatty acids (in particular butyrate) appear to regulate the expression of genes involved in controlling the proliferation, differentiation and apoptosis of intestinal epithelial cells [86]. Furthermore, VFA are the main and favourite metabolic substrate of colonocytes, providing them with approximately 60–70% of the energy requirements necessary for proliferation and differentiation in the equine colonic mucosa [87]. We must also remember that although faecal VFA concentration may not represent the actual caecal levels of VFA, they can provide indirect information about changes in VFA production [88]. Julliand et al. [89] reported that the concentration of fibrolytic bacteria and acetate production were lower in horses fed a diet based on hay and barley compared with a diet of hay only. In our study we saw an increase in acetate production in the NUCL group, which may explain a better adaptation of foals to a fibrous diet compared with the CTRL group. In general, in the foals receiving the nucleotide supplementation we observed a higher concentration of the main VFAs normally produced by the adult horse (namely, acetic acid, propionic acid and butyric acid) [90] compared with the CTRL group. This difference may mean that bacterial colonization of the hindgut and subsequent VFA production had occurred earlier in foals receiving nucleotides compared with untreated foals. This may mean that the foals receiving the nucleotides were able to adapt more readily to the dietary changes associated with the start of weaning. Another aspect that should be considered is the concentrations of butyric acid in faeces of foals treated with nucleotides. In particular, the concentration of faecal butyrate was higher in the foals of the NUCL group compared with in those receiving the placebo. This can be considered a positive aspect as butyric acid is a final

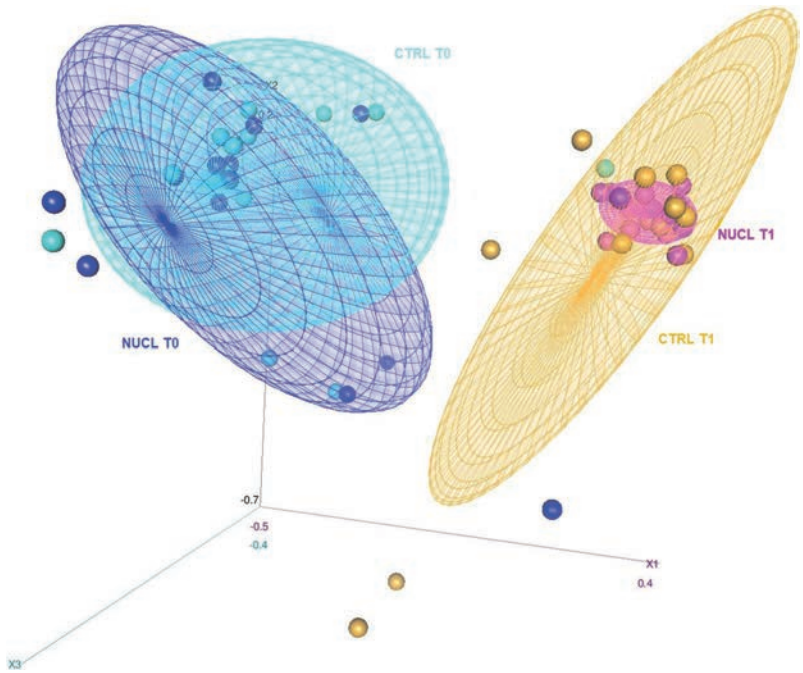

Fig. 9 3D PCoA plot with ellipse showing microbiota clusters of foals divided according to treatment group (Jaccard MetaMDS). Each dot represents the bacterial microbiota of a foal at T0 or T1. The NUCL T0 and CTRL T0 clusters are distinct to those for NUCL T1 and CTRL T1, and NUCL T1 samples are more closely clustered compared with those for CTRL T1

product of the microbial fermentation of fibre, and some studies have shown how it positively influences the health of the intestine, promoting the differentiation of colonocytes, exerting an anti-inflammatory effect and modulating oxidative stress [91]. Thus, the greater concentration of faecal butyrate in the foals of the NUCL group could be considered an index of intestinal well-being, in particular of enterocyte health, thus also explaining the decrease in the incidence of diarrhoea in these foals. Finally, the higher level of iso-butyric acid in the foals receiving the nucleotide supplement may also benefit their health, being involved in the degradation of the plant cell wall, promoting their digestion [92].

The main limitations in this study were due to the relatively small number of faecal samples for the microbiota analysis and the lack of assessment of the mares microbiota for comparison. In addition, the lack of a vaccination challenge and the short duration of the trial may have concealed longstanding effects of nucleotide supplementation.

Conclusions

The management of diarrhoea in foals presents a difficult task for both owners and equine veterinarians. As such, it is important to find new approaches to prevent and minimize these events. The use of dietary nucleotides in this study successfully decreased the incidence and gravity of diarrhoea episodes in new-born foals, as already demonstrated in other species. In addition, the increased growth rate recorded for the supplemented group and the beneficial changes noted in the gut microbiota further support the validity of using dietary nucleotides in these animals.

Abbreviations

BW	Body Weight
CTRL	Control group
DM	Dry matter
GC	Girth Circumference
HPLC	High Performance Liquid Chromatography
NUCL	Nucleotide group
PCoA	Principal Coordinate Analysis
SEM	Standard Error of the Mean
VFA	Volatile Fatty Acids

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13620-025-00294-3.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

The authors would like to acknowledge the breeding centres involved in the study, namely: "Centro Equino Arcadia", "Centro Equino delle Stelle" and "Centro Equino Le Fontanette" for their willingness to participate in the study. The authors would also like to thank the veterinary science students (Ms. Virginia

Tufarelli and Ms. Silvia Bravin) for their valuable contributions during sample collection and analysis.

Authors' contributions
LPe and LPr designed the study. LPr and PGT supervised this study. LPe performed the sample collection, analyzed the data and was the major contributor in writing the manuscript. EP contributed to sample collection and data analysis. TN, AB and EV contributed to the selection of foals, the creation of the work timeline and manuscript drafting. UA performed the statistical data processing and analysis. GR contributed to the selection of foals, the inclusion criteria and supplement administration. PGT and JH analyzed and interpreted the data regarding the faecal bacterial microbiota and contributed to the drafting of the manuscript in relation to the microbiota analysis. SB and GP performed the blood sample analyses. SA and LC performed the VFA analyses. All authors have read and approved the final manuscript.

Funding
This project was completely funded by the University of Turin research sector.

Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of the University of Turin (Ethical approval prot. n. 567, University of Turin). The owner of each breeding centre provided their written informed consent to participation to the study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Veterinary Sciences, University of Turin, Largo Braccini 2, Grugliasco 10095, Italy. [2]Centro Equino Arcadia, Frazione Mottura 106, Villafranca Piemonte 10068, Italy. [3]Department of Veterinary Medicine, University of Teramo, SP18 Piano d'Accio, Teramo 64100, Italy. [4]Istituto Zooprofilattico Sperimentale Piemonte, Liguria E Valle d'Aosta, Via Bologna 148, Turin 10154, Italy. [5]Consiglio Nazionale Delle Ricerche, Largo Braccini 2, Grugliasco 10095, Italy.

Received: 15 July 2024 Accepted: 19 February 2025
Published online: 01 March 2025

References

1. Schoster A, Staempfli HR, Guardabassi LG, Jalali M, Weese JS. Comparison of the fecal bacterial microbiota of healthy and diarrheic foals at two and four weeks of life. BMC Vet Res. 2017;13:144.
2. Oliver-Espinosa O. Foal diarrhea. Vet Clinics of North America: Equine Practice. 2018;34:55–68.
3. Magdesian KG. Neonatal foal diarrhea. Veterinary Clinics of North America: Equine Practice. 2005;21:295–312.
4. Mallicote M, House AM, Sanchez LC. A review of foal diarrhoea from birth to weaning. Equine Veterinary Education. 2012;24:206–14.
5. Kuhl J, Winterhoff N, Wulf M, Schweigert FJ, Schwendenwein I, Bruckmaier RM, et al. Changes in faecal bacteria and metabolic parameters in foals during the first six weeks of life. Vet Microbiol. 2011;151:321–8.
6. Johnston RH, Kamstra LD, Kohler PH. Mares' milk composition as related to "foal heat" scours. J Anim Sci. 1970;31:549–53.
7. Masri MD, Merritt AM, Gronwall R, Burrows CF. Faecal composition in foal heat diarrhoea. Equine Vet J. 1986;18:301–6.
8. Costa MC, Weese JS. Understanding the intestinal microbiome in health and disease. Veterinary Clinics: Equine Practice. 2018;34:1–12.
9. John J, Roediger K, Schroedl W, Aldaher N, Vervuert I. Development of intestinal microflora and occurrence of diarrhoea in sucking foals: effects of Bacillus cereus var. toyoi supplementation. BMC Vet Res. 2015;11:34.
10. De La Torre U, Henderson JD, Furtado KL, Pedroja M, Elenamarie O, Mora A, et al. Utilizing the fecal microbiota to understand foal gut transitions from birth to weaning. PLoS One. 2019;14: e0216211.
11. Goodman-Davis R, Figurska M, Cywinska A. Gut microbiota manipulation in foals—naturopathic diarrhea management, or unsubstantiated folly? Pathogens. 2021;10: 1137.
12. Yuyama T, Yusa S, Takai S, Tsubaki S, Kado Y, Morotomi M. Evaluation of a host-specific Lactobacillus Probiotic in neonatal foals. Int J Appl Res Vet Med. 2004;2:26–33.
13. Sadet-Bourgeteau S, Julliand V. Equine microbial gastro-intestinal health. In: Coenen M, Miraglia N, editors. Longland A. Ellis A. The Impact of nutrition on the health and welfare of horses. EAAP Publications. Cirencester: Wageningen Academic Publishers; 2010. p. 161–82.
14. Ströbel C, Günther E, Romanowski K, Büsing K, Urubschurov V, Zeyner A. Effects of oral supplementation of probiotic strains of *Lactobacillus rhamnosus* and *Enterococcus faecium* on diarrhoea events of foals in their first weeks of life. J Anim Physiol Anim Nutr. 2018;102:1357–65.
15. Urubschurov V, Stroebel C, Günther E, Romanowski K, Büsing K, Zeyner A. Effect of oral supplementation of probiotic strains of Lactobacillus rhamnosus and Enterococcus faecium on the composition of the faecal microbiota of foals. J Anim Physiol Anim Nutr. 2019;103:915–24.
16. Weese JS, Rousseau J. Evaluation of Lactobacillus pentosus WE7 for prevention of diarrhea in neonatal foals. J Am Vet Med Assoc. 2005;226:2031–4.
17. Schoster A, Staempfli HR, Abrahams M, Jalali M, Weese JS, Guardabassi L. Effect of a probiotic on prevention of diarrhea and Clostridium difficile and Clostridium perfringens shedding in foals. J Vet Intern Med. 2015;29:925–31.
18. Schoster A, Guardabassi L, Staempfli HR, Abrahams M, Jalali M, Weese JS. The longitudinal effect of a multi-strain probiotic on the intestinal bacterial microbiota of neonatal foals. Equine Vet J. 2016;48:689–96.
19. Costa MC, Stämpfli HR, Allen-Vercoe E, Weese JS. Development of the faecal microbiota in foals. Equine Vet J. 2016;48:681–8.
20. Lindenberg F, Krych L, Kot W, Fielden J, Frøkiær H, van Galen G, et al. Development of the equine gut microbiota. Sci Rep. 2019;9:14427.
21. Garber A, Hastie P, Murray J-A. Factors influencing equine gut microbiota: current knowledge. J Equine Vet. 2020;88: 102943.
22. Gil A. Modulation of the immune response mediated by dietary nucleotides. Eur J Clin Nutr. 2002;56:S1–4.
23. Ding T, Song G, Liu X, Xu M, Li Y. Nucleotides as optimal candidates for essential nutrients in living organisms: a review. Journal of Functional Foods. 2021;82: 104498.
24. Qu Z, Tian P, Zhao J, Wang G, Chen W. Feeding the microbiota–gut–brain axis: nucleotides and their role in early life. Food Frontiers. 2023;4:1164–78.
25. Carver JD, Allan WW. The role of nucleotides in human nutrition. J Nutr Biochem. 1995;6:58–72.
26. Fontana L, Martínez-Augustin O, Gil Á. Role of dietary nucleotides in immunity. Functional Food Reviews. 2010;2:91–100.
27. Carver JD. Dietary nucleotides: effects on the immune and gastrointestinal systems. Acta Paediatr. 1999;88:83–8.
28. Oftedal OT, Hintz HF, Schryver HF. Lactation in the horse: milk composition and intake by foals. J Nutr. 1983;113:2096–106.
29. Acworth NRJ. The healthy neonatal foal: routine examinations and preventative medicine. Equine Veterinary Education. 2003;15:207–11.
30. Knottenbelt DC, Holdstock N, Madigan JE. Equine neonatal medicine and surgery: medicine and surgery. Amsterdam: Elsevier Health Sciences; 2004.
31. Bonelli F, Nocera I, Conte G, Panzani D, Sgorbini M. Relation between Apgar scoring and physical parameters in 44 newborn Amiata donkey foals at birth. Theriogenology. 2020;142:310–4.
32. Alonso MA, Boakari YL, Riccio AV, Belli CB, Fernandes CB. Perinatal parameters of mule and equine foals: similarities and differences. Journal of Veterinary Behavior. 2023;63:31–5.
33. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–7.

34. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3.
35. Katoh K, Misawa K, Kuma K, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast fourier transform. Nucleic Acids Res. 2002;30:3059–66.
36. Price MN, Dehal PS, Arkin AP. FastTree 2 – approximately maximum-likelihood trees for large alignments. PLoS One. 2010;5:e9490.
37. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, et al. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 2007;35:7188–96.
38. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:D590–6.
39. Guantario B, Giribaldi M, Devirgiliis C, Finamore A, Colombino E, Capucchio MT, et al. A comprehensive evaluation of the impact of bovine milk containing different beta-casein profiles on gut health of ageing mice. Nutrients. 2020;12: 2147.
40. R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2021. URL http://www.R-project.org/.
41. Faith DP. Conservation evaluation and phylogenetic diversity. Biol Cons. 1992;61:1–10.
42. Pielou EC. The measurement of diversity in different types of biological collections. J Theor Biol. 1966;13:131–44.
43. Chao A, Chiu CH. Species estimation and applications. Wiley StatsRef: Statistics Reference Online. 2016. https://doi.org/10.1002/9781118445112.stat03432.pub2. Available from:
44. Shannon CE. A mathematical theory of communication. The Bell System Technical Journal. 1948;27:379–423.
45. Simpson EH. Measurement of diversity. Nature. 1949;163:688–688.
46. Lozupone CA, Hamady M, Kelley ST, Knight R. Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. Appl Environ Microbiol. 2007;73:1576–85.
47. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005;71:8228–35.
48. Bray JR, Curtis JT. An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr. 1957;27:326–49.
49. Jaccard P. The distribution of the flora in the alpine zone. 1. New Phytologist. 1912;11:37–50.
50. Anderson MJ. Permutational multivariate analysis of variance (PERMANOVA). Wiley StatsRef: Statistics Reference Online. John Wiley & Sons, Ltd; 2017. p. 1–15. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/9781118445112.stat07841. Cited 2023 Dec 10.
51. Bauer JE, Harvey JW, Asquith RL, McNulty PK, Kivipelto J. Serum protein reference values in foals during the first year of life: comparison of chemical and electrophoretic methods. Veterinary Clinical Pathology. 1985;14:14–22.
52. Gueimonde M, Sánchez B, de los Reyes-Gavilán C, Margolles A. Antibiotic resistance in probiotic bacteria. Front Microbiol. 2013;4. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2013.00202. Cited 2022 Nov 18.
53. Wohlfender FD, Barrelet FE, Doherr MG, Straub R, Meier HP. Diseases in neonatal foals. Part 1: the 30 day incidence of disease and the effect of prophylactic antimicrobial drug treatment during the first three days post partum. Equine Vet J. 2009;41:179–85.
54. Schoster A. Probiotic use in equine gastrointestinal disease. Vet Clin North Am Equine Pract. 2018;34:13–24.
55. Pickering LK, Granoff DM, Erickson JR, Masor ML, Cordle CT, Schaller JP, et al. Modulation of the immune system by human milk and infant formula containing nucleotides. Pediatrics. 1998;101:242–9.
56. Carver JD, Pimentel B, Cox WI, Barness LA. Dietary nucleotide effects upon immune function in infants. Pediatrics. 1991;88:359–63.
57. Sanderson IR, He Y. Nucleotide uptake and metabolism by intestinal epithelial cells. J Nutr. 1994;124:131S–137S.
58. Savaiano DA, Clifford AJ. Adenine, the precursor of nucleic acids in intestinal cells unable to synthesize purines de novo. J Nutr. 1981;111:1816–22.
59. LeLeik NS, Bronstein AD, Baliga BS, Munro HN. De novo purine nucleotide synthesis in the rat small and large intestine: effect of dietary protein and purines. J Pediatr Gastroenterol Nutr. 1983;2:313–9.
60. Cosgrove M. Nucleotides. Nutrition. 1998;14:748–51.
61. Uauy R, Stringel G, Thomas R, Quan R. Effect of dietary nucleosides on growth and maturation of the developing gut in the rat. J Pediatr Gastroenterol Nutr. 1990;10:497–503.
62. Van Buren CT, Rudolph F. Dietary nucleotides: a conditional requirement : is nucleic acid actually a nutrient? Nutrition. 1997;13:470–2.
63. Kulkarni AD, Rudolph FB, Van Buren CT. The role of dietary sources of nucleotides in immune function: a review. J Nutr. 1994;124:1442S–1446S.
64. Carver JD, Cox WI, Barness LA. Dietary nucleotide effects upon murine natural killer cell activity and macrophage activation. J Parenter Enter Nutr. 1990;14:18–22.
65. Yamauchi K, Adjei AA, Ameho CK, Chan YC, Kulkarni AD, Sato S, et al. A nucleoside-nucleotide mixture and its components increase lymphoproliferative and delayed hypersensitivity responses in mice. J Nutr. 1996;126:1571–7.
66. Nagafuchi S, Katayanagi T, Nakagawa E, Takahashi T, Yajima T, Yonekubo A, et al. Effects of dietary nucleotides on serum antibody and splenic cytokine production in mice. Nutr Res. 1997;17:1163–74.
67. Nagafuchi S, Totsuka M, Hachimura S, Goto M, Takahashi T, Yajima T, et al. Dietary nucleotides increase the proportion of a TCRγδ+ subset of intraepithelial lymphocytes (IEL) and IL-7 production by intestinal epithelial cells (IEC); implications for modification of cellular and molecular cross-talk between IEL and IEC by dietary nucleotides. Biosci Biotechnol Biochem. 2000;64:1459–65.
68. Nagafuchi S, Hachimura S, Totsuka M, Takahashi T, Goto M, Yajima T, et al. Dietary nucleotides can up-regulate antigen-specific Th1 immune responses and suppress antigen-specific IgE responses in mice. Int Arch Allergy Immunol. 2000;122:33–41.
69. Nagafuchi S, Totsuka M, Hachimura S, Goto M, Takahashi T, Yajima T, et al. Dietary nucleotides increase the mucosal IgA response and the secretion of transforming growth factor β from intestinal epithelial cells in mice. Cytotechnology. 2002;40:49–58.
70. Jyonouchi H, Sun S, Abiru T, Winship T, Kuchan MJ. Dietary nucleotides modulate antigen-specific type 1 and type 2 t-cell responses in young c57bl/6 mice. Nutrition. 2000;16:442–6.
71. Jyonouchi H, Sun S, Winship T, Kuchan MJ. Dietary ribonucleotides modulate type 1 and type 2 T-helper cell responses against ovalbumin in young BALB/cJ mice. J Nutr. 2001;131:1165–70.
72. Buck RH, Thomas DL, Winship TR, Cordle CT, Kuchan MJ, Baggs GE, et al. Effect of dietary ribonucleotides on infant immune status. Part 2: immune cell development. Pediatr Res. 2004;56:891–900.
73. Schaller JP, Kuchan MJ, Thomas DL, Cordle CT, Winship TR, Buck RH, et al. Effect of dietary ribonucleotides on infant immune status. Part 1: humoral responses. Pediatr Res. 2004;56:883–90.
74. Ostrom KM, Cordle CT, Schaller JP, Winship TR, Thomas DJ, Jacobs JR, et al. Immune status of infants fed soy-based formulas with or without added nucleotides for 1 year: part 1: vaccine responses, and morbidity. J Pediatr Gastroenterol Nutr. 2002;34:137.
75. Lee DN, Liu SR, Chen YT, Wang RC, Lin SY, Weng CF. Effects of diets supplemented with organic acids and nucleotides on growth, immune responses and digestive tract development in weaned pigs. J Anim Physiol Anim Nutr. 2007;91:508–18.
76. Sauer N, Eklund M, Bauer E, Gänzle MG, Field CJ, Zijlstra RT, et al. The effects of pure nucleotides on performance, humoral immunity, gut structure and numbers of intestinal bacteria of newly weaned pigs. J Anim Sci. 2012;90:3126–34.
77. Superchi P, Saleri R, Borghetti P, Angelis E, Ferrari L, Cavalli V, et al. Effects of dietary nucleotide supplementation on growth performance and hormonal and immune responses of piglets. Animal : an international journal of animal bioscience. 2012;6:902–8.
78. Cameron BF, Wong CW, Hinch GN, Singh D, Nolan JV, Colditz IG. Effects of nucleotides on the immune function of early-weaned piglets. In: Digestive physiology of pigs proceedings of the 8th Symposium, Swedish University of Agricultural Sciences, Uppsala, Sweden, 20–22 June 2000. 2001. p. 66–8.
79. Jang KB, Kim SW. Supplemental effects of dietary nucleotides on intestinal health and growth performance of newly weaned pigs. J Anim Sci. 2019;97:4875–82.
80. Cosgrove M, Davies DP, Jenkins HR. Nucleotide supplementation and the growth of term small for gestational age infants. Archives of Disease in Childhood - Fetal and Neonatal Edition. 1996;74:F122–5.

81. Singhal A, Macfarlane G, Macfarlane S, Lanigan J, Kennedy K, Elias-Jones A, et al. Dietary nucleotides and fecal microbiota in formula-fed infants: a randomized controlled trial. Am J Clin Nutr. 2008;87:1785–92.
82. Liu Y, Xie C, Zhai Z, Deng Z, Jonge HRD, Wu X, et al. Uridine attenuates obesity, ameliorates hepatic lipid accumulation and modifies the gut microbiota composition in mice fed with a high-fat diet. Food Funct. 2021;12:1829–40.
83. Boutard M, Cerisy T, Nogue P-Y, Alberti A, Weissenbach J, Salanoubat M, et al. Functional diversity of carbohydrate-active enzymes enabling a bacterium to ferment plant biomass. PLoS Genet. 2014;10: e1004773.
84. Wunderlich G, Bull M, Ross T, Rose M, Chapman B. Understanding the microbial fibre degrading communities, processes in the equine gut. Anim microbiome. 2023;5:3.
85. Li N, Yan F, Wang N, Song Y, Yue Y, Guan J, et al. Distinct gut microbiota and metabolite profiles induced by different feeding methods in healthy Chinese infants. Front Microbiol. 2020;11:11.
86. Geor RJ, Harris PA. How to minimize gastrointestinal disease associated with carbohydrate nutrition in horses. In: Proceedings of the annual convention of the AAEP. 2007. p. 178–85.
87. Vermorel M, Martin-Rosset W. Concepts, scientific bases, structure and validation of the French horse net energy system (UFC). Livest Prod Sci. 1997;47:261–75.
88. Berg EL, Fu CJ, Porter JH, Kerley MS. Fructooligosaccharide supplementation in the yearling horse: effects on fecal pH, microbial content, and volatile fatty acid concentrations. J Anim Sci. 2005;83:1549–53.
89. Julliand V, de Fombelle A, Drogoul C, Jacotot E. Feeding and microbial disorders in horses: part 3—effects of three hay:grain ratios on microbial profile and activities. J Equine Vet. 2001;21:543–6.
90. Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev. 1990;70:567–90.
91. Whitfield-Cargile CM, Chung HC, Coleman MC, Cohen ND, Chamoun-Emanuelli AM, Ivanov I, et al. Integrated analysis of gut metabolome, microbiome, and exfoliome data in an equine model of intestinal injury. Microbiome. 2024;12:74.
92. Gorosito AR, Russell JB, Van Soest PJ. Effect of carbon-4 and carbon-5 volatile fatty acids on digestion of plant cell wall in vitro1. J Dairy Sci. 1985;68:840–7.

Publisher's Note