

DIFFERENCES IN GUT MICROFLORA BETWEEN CAPTIVE AND WILD BIRDS: ARE WE GETTING THE CAPTIVE BALANCE RIGHT?

C.J. Minson, BSC,^{1*} R.G. Lentle, PhD, MD,² M.A. Potter, Ph³

¹Postgraduate Diploma; Ecology Department, INR, Massey University, Palmerston North, New Zealand; ²Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand; ³Ecology Department, INR, Massey University, Palmerston North, New Zealand

Abstract

The microbiota of the vertebrate gastrointestinal tract consists of a diverse collection of microbial species.⁴ In the past, identification of these species has involved cultivation-based techniques. However, due to the dependence upon bacteria-specific media during cultivation, up to 80% of species may not have been identified using these techniques.^{5,6} To overcome this bias, a DNA-based technique of identifying microbial communities is now routinely employed. Denaturing Gradient Gel Electrophoresis (DGGE) uses 16S rDNA as a molecular fingerprint to identify species. Sections of DNA fragments are amplified using polymerase chain reactions (PCR), then loaded into a gel containing a chemical gradient.³ Depending upon the sequence of base pairs in the fragment the DNA will ‘melt’ (denature) at a specific gradient causing a band to appear on the gel. Each band corresponds to a different bacterial species, thus providing a profile of the bacterial community for that sample.

Both the quality of diet ingested and the composition of microflora within the gastrointestinal tract exert an important influence over the overall health of an animal.¹ The structure of the microbial community within the digestive tract alters with a change in diet,² thus highlighting the role specific bacteria play in digestion, particularly for the breakdown of less digestible dietary components, for example, plant material or chitin. The microbiota of the gastrointestinal tract has been widely studied in the more common or economically important species, such as humans or poultry, it is less well understood in wild animals, particularly non-mammalian species. Furthermore, there is no published literature on the difference in the structure of the microbial community within the digestive tract of captive aves compared to their wild counterparts. Since bacteria play an integral part in the breakdown of dietary items for nutrient uptake as well as in overall immunity, both quality of digestion and immune status must be affected by the structure of the microbial community. If community structure is dictated by diet, then wild animals feeding on a natural diet may have a different suite of intestinal bacteria than captive animals. This factor needs to be addressed when hard-releasing captive animals into the wild, as the inability to immediately digest a natural diet may severely affect post-release survival.

This study looked at the difference in the structure of bacterial communities within the gastrointestinal tract of three native New Zealand avian species, North Island brown kiwi (*Apteryx mantelli*), brown teal (*Anas chlorotis*) and takahe (*Porphyrio* [Notornis] *mantelli*). Representatives from both wild-living and captive birds were included. Faecal samples from each bird were collected, DNA extracted, PCR conducted and replicated DNA fragments loaded into a denaturing gradient gel. Gel bands were analysed with Phoretics and analyses of variance were conducted on total band number per individual, and Simpson’s and Shannon Weiner Indices using MinitabTM 15.1.0.0.⁷

A greater number of bacterial species were found in birds held in captivity than those found in the wild, irrespective of species (Table 1). No difference was found in the diversity of bacteria among species. These findings have an important bearing on the protocol for release of captive-held species into the wild. Greater emphasis may need to be given to gradually changing the diet fed in captivity into a more natural one before animals are released. This may increase the ability of newly released animals to digest a wild diet, thus decreasing post-release weight loss and increasing overall survival.

LITERATURE CITED

1. Amit-Romach, E., D. Sklan, and Z. Uni. 2004. Microflora ecology of the chicken intestine using 16S Ribosomal DNA primers. *Poultry Science* 83:1093-1098.
2. Hill, M.J. 1981. Diet and the Human Intestinal Bacterial Flora. *Cancer Research* 41:3778-3780.
3. Hill, J.G III, I. Hanning, S.J. Beaupre, S.J. Ricke, and M.M. Slavik. 2008. Denaturing gradient gel electrophoresis for the determination of bacterial species diversity in the gastrointestinal tracts of two crotaline snakes. *Herpetological Review* 39(4):433-438.
4. Santos, A.A. Jr., P.R. Ferket, F.B.O. Santos, N. Nakamura, and C. Collier. 2008. Change in the ileal bacterial population of turkeys fed different diets and after infection with *Salmonella* as determined with denaturing gradient gel electrophoresis of amplified 16S ribosomal DNA. *Poultry Science* 87:1415-1427.
5. Suau, A., R. Bonnet, M. Sutren, J.J. Gordon, G.R. Gibson, M.D. Collins, and J. Dore. 1999. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Applied Environmental Microbiology* 65:4799-4807.
6. Vaughan, E.E., F. Schut, H.G.H.J. Helig, E.G.W.M. de Vos, E. G. W. M. and A.D.L. Akkermans. 2000. A molecular view of the intestinal ecosystem. *Current Issues in Intestinal Microbiology* 1:1-12.
7. MiniTab. 2006. MiniTab, Rel. 15.1.0. 2006.

Table 1. Analyses of variance of band number, Simpson's and Shannon Weiner Indices of captive and wild North Island brown kiwi, takahe and brown teal. Johnson Transformations were used where necessary to normalise data.

Species	Comparisons	Analysis type	Pvalue	Mean (SE) (*Data back transformed where necessary)
Combined data from all species (kiwi, takahe, brown teal)	Combined species (captive + wild)	Band number	P = 0.524	
		Simpson's Index	P = 0.674	
		Shannon Weiner Index	P = 0.465	
	captive vs wild	Band number	P = 0.018	captive = 14.64 (SE = 1.00) wild = 10.39 (SE = 1.42)
		Simpson's Index	P = 0.036	captive = 0.82 (SE = 0.02)* wild = 0.74 (SE = 0.29)*
		Shannon Weiner Index	P = 0.043	captive = 1.30×10^6 (SE = 1.85×10^5)* wild = 7.7×10^5 (SE = 1.9×10^5)*