EFFECTS OF LACTOBACILLUS TREATMENTS ON THE GUT FLORA OF JUVENILES AND ADULT BLUE MACAWS (CYANOPSITTA SPIXII, ANODORHYNCHUS LEARI, ANODORHYNCHUS HYACINTHINUS) AT AL WABRA WILDLIFE PRESERVATION (AWWP), QATAR

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Summary

Evaluation of faecal samples in captive birds has potential as a non-invasive monitoring tool for early detection of gastro-intestinal problems. Most blue macaws held at Al Wabra Wildlife Preservation (AWWP) undergo regular health monitoring that includes a monthly faecal sample evaluation. Birds in which the bacterial floral abundance and balance did not meet the described values for parrots (RITCHIE et al., 1994) were supplemented with lactobacilli. In this study, faecal samples before and after lactobacilli treatment were collected from 47 adult spix’s macaws and 16 juvenile birds (9 spix’s, 4 lear’s and 3 hyacinth macaws). A significant difference in the ratio of gram-positive rods to gram-positive cocci before and after treatment was found. This study demonstrates that lactobacillus feeding does improve the ratio of gram-positive rods to gram-positive cocci, but has no influence on the abundance of bacteria.

Introduction

The use of probiotics in the management of birds is under debate. In humans, lactobacilli as probiotics are common additives in food products to stimulate the immune response (Doron and Sherwood, 2006). In poultry, several reviews have emphasised potentially positive effects of probiotics on the performance and immune status or immune response of the animals (Mead, 2000; Patterson and Burkholder, 2003; Chichlowski et al., 2007). However, in several studies, no positive effects of probiotics on performance or parameters of the immune status could be demonstrated (SIRIKEN et al., 2003, Ribeiro et al., 2007). For pet birds or wild birds kept in captivity at zoological institutions, the use of probiotics is sometimes promoted (RITCHIE et al., 1994), although specific studies are lacking. Three species of rare blue macaws, Spix’s, Lear’s and hyacinth macaws, are held at Al Wabra Wildlife Preservation (AWWP). Lactobacilli feeding as probiotics are used to support the health of these threatened species.

The Spix’s macaw (Cyanopsitta spixii) is the only member of the genus Cyanopsitta and has been presumed extinct in the wild since around the year 2000, due to loss of habitat and illegal trapping (Del Hoyo et al., 1997). At the time of writing, there were 63 individuals in the International studbook managed captive population, distributed in Brazil, Switzerland, Tenerife and the Al Wabra Wildlife Preservation (AWWP), Qatar (Watson et al., 2008). Forty-seven animals are held at the Al Wabra Wildlife Preservation (AWWP) where 16 new individuals have been bred so far (Watson et al., 2007). Intensive preventive health management of Spix’s macaws is an important tool in the conservation effort for this species.
The Lear’s macaw (*Anodorhynchus leari*) is critically endangered, which means that this species is at high risk of extinction in the wild (IUCN, 2008). There is only a small population left in Brazil, which is currently believed to be increasing thanks to a constant presence of biologists at the 2 sites at which they breed. However, should this monitoring ever stop, they are likely to decline again because of trapping for illegal trade (IBAMA, 2006).

The hyacinth macaw (*Anodorhynchus hyacinthinus*) is classified as endangered and is protected under Brazil law as well as being banned from export in all countries of origin (Capper et al., 2004). Most of the remaining population is located in Brazil but there are also small numbers in Paraguay and Bolivia (Snyder et al., 2000). Thanks to conservation efforts their numbers are increasing in some areas but likely declining in other areas where there is no regular population monitoring or efforts to protect them against trapping, and where habitat loss continues unabated (Gueded, 2004).

**Materials and methods**

A monitoring system was established at AWWP to detect imbalances in gut flora at an early stage, and to allow early treatment before the occurrence of clinical problems. Early or prophylactic treatment consisted of the application of a lactobacillus product (*Lactobacillus salivarius*; product: PT-12, company: Rescha, 33142 Büren, Germany) developed for parrots. The aim of this study was a retrospective evaluation of the data from faecal sampling to test whether a supposedly beneficial influence of the lactobacillus treatment on faecal bacterial parameters could be demonstrated.

Ritchie et al. (1994) described that the normal gut flora of psittacines should contain 100 to 200 bacteria per microscope field (magnification 1,000 x) with 60 to 80 % gram-positive rods and 20 to 40 % of gram-positive cocci. Adult Spix’s macaws ($n = 47$) were tested once a month within the intensive preventive health management programme. Samples from hand-reared juvenile macaws (Spix’s ($n = 9$), Lear’s ($n = 4$), hyacinth macaws ($n = 3$)) were routinely collected at days 3 and 7 after hatching and then once a week until weaning, or upon request if they presented with clinical signs of illness. Before March 2007, samples from adult birds were collected at any time of the day. After March 2007, samples were collected consistently in the morning only, since a previous study conducted at AWWP had established that time of collection of faeces had a profound influence of bacterial abundance in flora, and that only early morning collection, preferably the first faecal of the day, provided samples with adequate bacteria per field to draw meaningful conclusions (Watson et al., 2007). Samples were not always collected systematically with respect to time before and after treatment, so for example; 1 bird had a sample analysed 2 days before treatment and 7 days after treatment, another bird 7 days before and 4 days after treatment etc., as this was dependent on the time availability of staff. All faecal samples were diluted and smears were prepared on microscope slides. The smears were stained by gram stain (oxalate crystal violet solution, stabilised lugol PVP, aceton alcohol, safranin solution; Biomérieux, 69280 Marcy l’Etoile, France) and examined under microscope (Motic 2000; Motic Deutschland GmbH, 35578 Wetzlau, Germany). The number of bacteria per microscopic field (magnification 1,000 x) was counted and the ratio of gram-positive rods to gram-positive cocci was estimated in a number of fields in different areas of the slide. Samples with a number of bacteria per microscopic field (magnification 1,000 x) lower than 100 or a ratio lower than 60:40 or higher as 80:20 gram-positive rods to gram-positive cocci were labelled, in accordance to Ritchie et al. (1994), as “imbalanced flora”. Those with a bacterial number higher than 100 and a ratio of gram-positive rods and cocci between 60:40 and 80:20 were considered “balanced flora”. Gram-negative bacteria and yeast were detected in only very few of the samples. Therefore, they are not evaluated in this study. If the values measured in the faeces were not within the range deemed normal by Ritchie et al. (1994), the corresponding birds received lactobacillus.
Lactobacillus feeding has also been used as a prophylactic treatment regularly in all AWWP Spix’s macaws. They received lactobacillus twice a year for 2 weeks (5 g/day for 47 birds), and in addition a double dose (10 g/day for 47 birds) for 2 days every 6 weeks.

From the available faecal bacteriology data, only the samples examined no more than 7 days before and 7 days after the lactobacillus treatment were included, resulting in 42 datasets from 30 individuals. The differences in the number of bacteria per microscope field (magnification 1,000 x) and the ratio of gram-positive rods to gram-positive cocci before and after the lactobacillus treatment were calculated. As the difference in the number of bacteria and in the ratio did not show a normal distribution, nonparametric tests (Wilcoxon test and sign test) were used to test if the different effects were significant. Tests were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The assumption was that lactobacillus feeding would increase the number of bacteria and the ratio of gram-positive rods to gram-positive cocci.

Results and discussion

Considering all data, there was no difference in the number of bacteria per microscopic field (magnification 1,000 x) before and after treatment (mean difference ± standard deviation 6 ± 265, p = 0.819), but the difference in the ratio of gram-positive rods to cocci was significant (14 ± 28, p = 0.005). The sign test was not significant for the difference in the number of bacteria, but the difference in the ratio of rods to cocci was (p = 0.006). The 7 birds with a balanced flora before treatment did not show any differences either in the number of bacteria (p = 0.916, figure 1) or in the ratio of rods to cocci (p = 0.796, figure 2). In the 35 birds where the flora was imbalanced before treatment, again no effect on the number of bacteria was found (p = 0.817), but there was an increased ratio of gram-positive rods to cocci (p = 0.002, sign test p = 0.004). Lactobacillus feeding of a bird with an imbalanced flora before treatment had a significant positive effect on the ratio of gram-positive rods to gram-positive cocci, but not on the number of bacteria.

![Figure 1: Number of bacteria per microscopic field (magnification 1000x) in the faecal samples of young and adult blue macaws (Cyanopsitta spixii, Anodorhynchus leari, Anodorhynchus hyacinthinus) before and after oral lactobacillus (L.Salivarius) treatment.](image)

However, the effect of time of collection, as established in the previous study at AWWP (Watson et al., 2007), on bacterial abundance in the faecal sample has a confounding effect on these results. Although the reasons behind this observation remain to be elucidated, perhaps a more effective evaluation of the lactobacillus treatment would be possible if only the faecal samples collected after March 2007 were evaluated.
It is, however, presumed that the ratio or balance of flora is more important than the number of bacteria per field, and in this study, lactobacillus appeared to have a positive effect in restoring balance in gut flora. The treatment of birds with balanced flora did not show negative effects, indicating that the feeding of lactobacillus did not destabilise the gut flora. Therefore, lactobacillus feeding was successful because the flora of birds with imbalanced flora before lactobacillus treatment improved, and the flora of birds with balanced flora was not negatively affected. To feed birds with lactobacillus thus appears to be an effective prophylaxis in restoring the stability of gut flora without the adverse effects often observed with antibiotics. It should be noted that the absence of clinical symptoms associated with dysbacteriosis like diarrhoea, vomiting, dehydration and apathy, in the animals monitored does not prove the efficacy of the prophylactic treatment; one can only conclude it did not do any harm. In order to produce more conclusive results, studies including a control group would have to be initiated.

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**References**


