INTESTINAL MICROBIOTA RESPONSES TO THE DIETARY SUPPLEMENTATION OF *BACILLUS* SP. PB6 (ATCC PTA-6737) AND FIBER LEVEL IN BROILER CHICKENS

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INTRODUCTION

The importance of beneficial microbes in maintaining intestinal health in broiler chickens is increasingly acknowledged. Dietary interventions are perhaps the most practical strategies to steer the microbiota under commercial conditions, either by feed additive supplementation or nutritional modulation. Microbiota responses to the supplementation of *Bacillus* sp. PB6 (ATCC PTA-6737) (PB6) have already been partly shown. In addition, the level of dietary fiber is another important factor that shapes intestinal microbial communities.

The objective of this study was to evaluate the microbiota responses to PB6 supplementation in the drinking water and to dietary fiber.

MATERIALS AND METHODS

The experiment was a 2 x 2 factorial design of PB6 supplementation or not at 1 x 10^8 CFU/liter (PB6), and of dietary fiber level (low fiber, LF; high fiber, HF). LF and HF diets respectively had crude fiber levels of 2.57% vs 3.57% (d0-10), 2.47% vs 3.66% (d10-21), and 2.39% vs 3.83% (d21-35).

Low fiber (LF) n=400	High fiber (HF) n=400	HF + PB6 n=400	LF + PB6 n=400

Male Ross 308 day-old chicks (n=1600) were randomly allocated to the 4 groups consisting of 10 pens of 40 birds each.

Cecal content was collected from 2 birds per pen on d28 for DNA extraction. Library preparation was performed after full-length 16S rRNA gene amplification followed by sequencing on a MinION Flow Cell using a GridION device (Oxford Nanopore Technologies, UK).

RESULTS

Alpha-diversity was higher for LF PB6+ birds at both genus (p=0.03) and species (p=0.04) level vs LF birds. PB6 addition increased α-diversity of LF birds to comparable levels to that of HF birds (**Fig. 1**).

On **phylum level**, LF + PB6 birds had more Firmicutes than LF birds (p=0.02). LF birds had more Proteobacteria than HF birds (p= 0.03), LF + PB6 birds (p=0.01) and HF + PB6 birds (p=0.02). LF birds had less Bacteroidetes than LF + PB6 (p<0.01), HF (p<0.01) and HF + PB6 birds (p=0.04) (**Fig. 2**).

At **family level**, fiber degraders *Christensenellaceae* and *Rikenellaceae*, were less abundant in LF compared to LF + PB6 (C p=0.02; R p<0.01), HF (C p=0.02; R p=0.01) and HF + PB6 birds (C p=0.01; R p=0.01). PB6 supplementation brought these 2 families to similar levels as HF groups. *Lachnospiraceae* were more abundant in HF than LF birds (p=0.04) and did not significantly differ from LF + PB6 birds. This showed again that adding PB6 to the LF diet favored a cecal microbiota resembling that of HF-fed birds (**Fig. 3**).

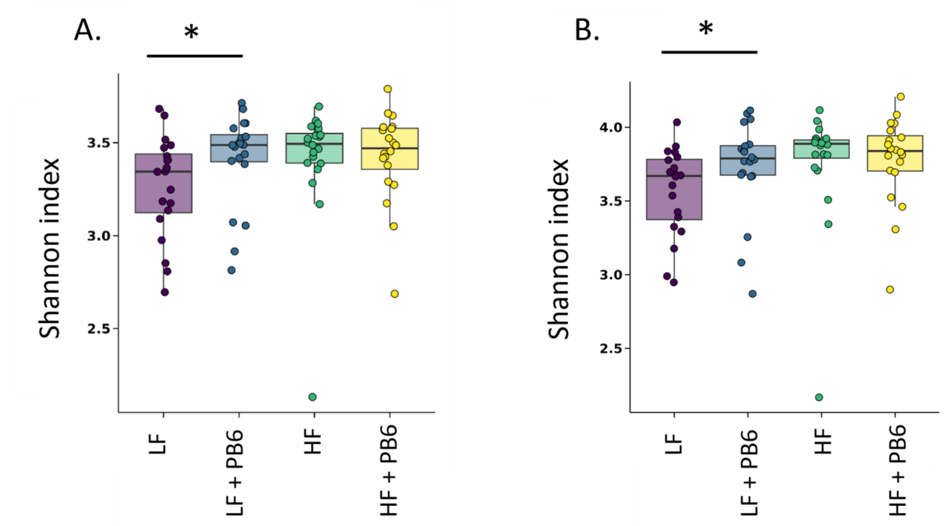

Fig. 1 | Effects of fiber level and PB6 supplementation on the alpha diversity of cecal content. **(A)** alpha diversity, as captured with the Shannon index on genus and **(B)** species level, respectively. LF: low fiber; HF: high fiber; PB6: probiotic supplementation; *: p-value < 0.05.

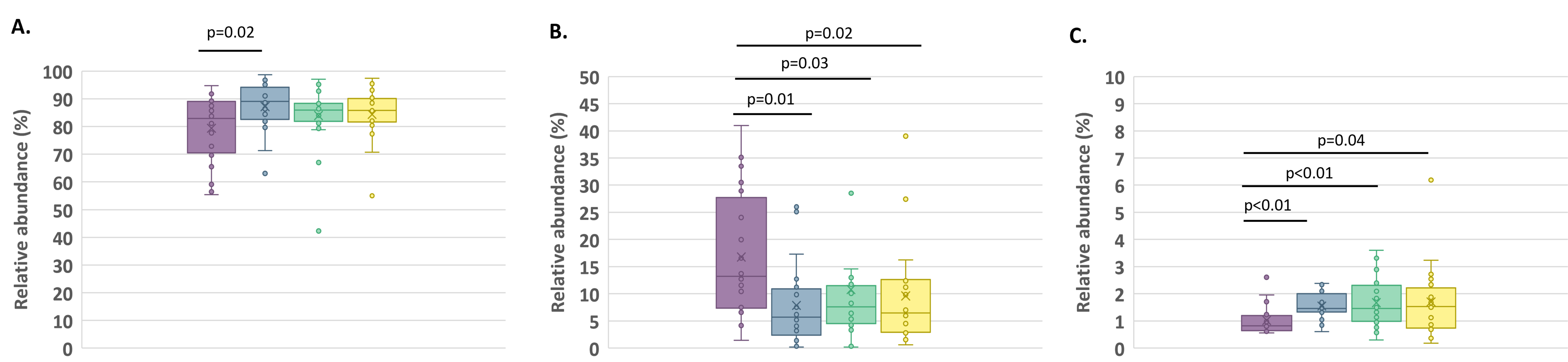

Fig. 2 | Effects of fiber level and PB6 supplementation on the relative abundance of **(A)** Firmicutes, **(B)** Proteobacteria, and **(C)** Bacteroidetes in cecal content of broilers receiving LF, LF + PB6, HF, and HF + PB6 diet, respectively.

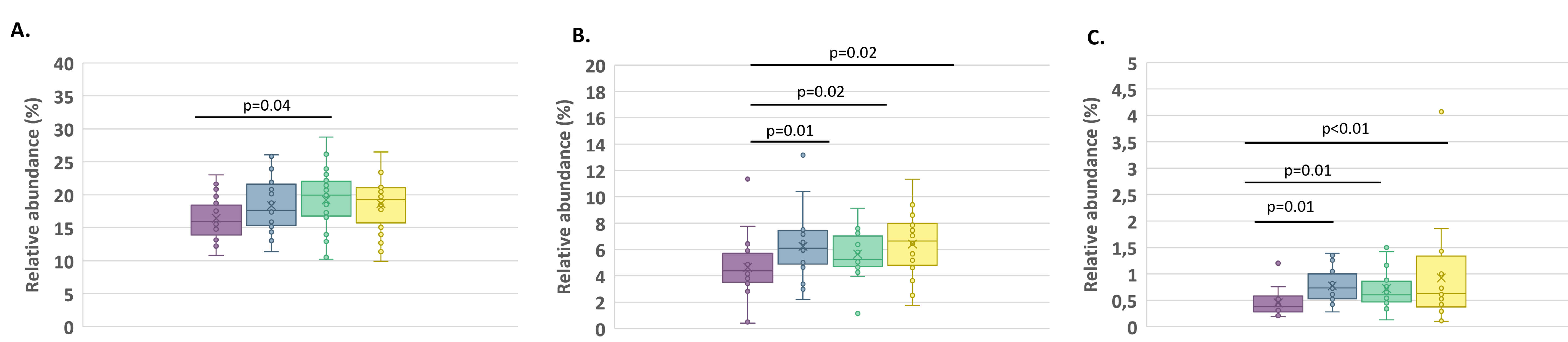

Fig. 3 | Effects of fiber level and PB6 supplementation on the relative abundance of **(A)** *Lachnospiraceae*, **(B)** *Christenellaceae*, and **(C)** *Rikenellaceae* in cecal content of broilers receiving LF, LF + PB6, HF, and HF + PB6 diet respectively.

CONCLUSION

This study showed that HF diet improved the diversity and composition of the cecal microbiota and that the changes due to LF could, at least partly, be restored by PB6 supplementation.

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