Overfeeding and genetics affect the composition of intestinal microbiota in Anas platyrhynchos (Pekin) and Cairina moschata (Muscovy) ducks

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duck microbial diversity; ileum; ceca; overfeeding; genotype; 454 pyrosequencing.

Abstract
To investigate the effect of overfeeding on the ileal and cecal microbiota of two genotypes of ducks (Pekin and Muscovy), high-throughput 16S rRNA gene-based pyrosequencing was used. The ducks were overfed for 12 days with 58% maize flour and 42% maize grain. Samples were collected before the overfeeding period (at 12 weeks), at 13 weeks, at 14 weeks, and 3 h after feeding. In parallel, ducks fed ad libitum were killed at the same ages. Whatever the digestive segment, the genotype, and the level of intake, Firmicutes and Bacteroidetes are the dominant phyla in the bacterial community of ducks (at least 80%). Before overfeeding, ileal samples were dominated by Bacilli, Clostridia, and Bacteroidia classes (≥70%), and cecal samples, by Bacteroidia and Clostridia classes (around 90%) in both Pekin and Muscovy ducks. The richness and diversity decreased in the ileum and increased in the ceca after overfeeding. Overfeeding triggers major changes in the ileum, whereas the ceca are less affected. Overfeeding increased the relative abundance of Clostridiaceae, Lactobacillaceae, Streptococcaceae, and Enterococcaceae families in the ileum, whereas genotype affects particularly three families: Lachnospiraceae, Bacteroidaceae, and Desulfovibrionaceae in the ceca.

Introduction
The intestinal microbiota plays a major role in animal’s health and host physiology (Backhed et al., 2004), for example, in immunological development or nutrient utilization (Gordon & Pesti, 1971). Microbiota interacts with the intestinal mucosa and can modify its structures and activity (Taschuk & Griebel, 2012). Different stresses experienced by animals such as a change in diet can modify the composition of the microbiota (Serino et al., 2011) as happens when overfeeding ducks during the fatty liver (‘foie gras’) production. During this period, the animals are overfed exclusively with maize, which is rich in carbohydrates (especially starch), to induce a hepatic steatosis resulting in the storage of fatty acids in the liver. This unbalanced diet could affect the equilibrium of the gut bacterial community. Indeed, the digestive tract is colonized by a large number of bacterial species. In chickens, the bacterial activity is more intense in the crop, the ceca, and small intestine in comparison with proventriculus gizzard and pancreas (Fuller, 1984). Most of the bacteria identified by clone library analysis belong to the phyla Bacteroidetes and Firmicutes (Zhu et al., 2002; Lu et al., 2003; Ahmed et al., 2008). Studies in classical microbiology and molecular biology show that the three segments of the small intestine contain facultative anaerobic bacteria, while the ceca contain strict anaerobic bacteria (Mead & Adams, 1975; Zhu et al., 2002; Lu et al., 2003). Lactobacilli are the major bacterial population in the small intestine (duodenum, jejenum, and ileum), whereas Clostridium spp. and Bacteroides spp. (obligate anaerobes) are dominant in the ceca with both...
The microbiota of several bird species has been studied by molecular analysis, but not in ducks (Kohl, 2012). The objective of this study was to identify the microbiota composition of two genotypes of ducks, Pekin (Anas platyrhynchos), which are used for their rapid growth and reproduction, and Muscovy (Cairina moschata), which have a high capacity for fat storage. It has been shown that during overfeeding, Pekin ducks tend to achieve significant storage in peripheral tissues, while the liver storage is less than in others species (Davail et al., 2003a; Hermier et al., 2003) and suggested that lipoprotein lipase (LPL), which hydrolyzes the lipoproteins rich in triacylglycerol, can partly explain lipid distribution between liver and hepatic tissues (Davail et al., 2003b). Interestingly, the LPL activity remains high during the overfeeding period in Pekin ducks and dramatically decreases in Muscovy ducks. This study is the first inventory of intestinal microbiota in ducks in both ileum and ceca. Furthermore, the genetic impact was evaluated using the two genotypes Muscovy and Pekin, and the effect of overfeeding to know the impact of a food stress on the microbiota was studied using a FLX amplicon pyrosequencing.

Material and methods

Experimental design

All experimental procedures involving ducks were in accordance with the French national guidelines for the care of animal for research purposes. Male ducks were raised in a breeding structure belonging to the French National Research Institute for Agronomy (INRA) at the ‘domaine d’Artiguères’, Benquet, France. From the first day of life to 4 weeks of age, they were fed ad libitum with 2-mm granules on a diet providing 11.93 MJ kg\(^{-1}\) of food and crude protein (CP) 17.5% (‘starter diet’). From 5 to 12 weeks of age, the birds were fed with 4-mm granules on a restricted diet providing 11.72 MJ kg\(^{-1}\) of food and CP 15.5%. The composition of the different feeds used in this study is listed in Table 1. At 12 weeks of age, all animals were weighed, and 25 ducks were selected. Of these, three of each genotype were killed before overfeeding. The remaining 12 animals (called overfed ducks) were fed in cages containing four ducks. They were hydraulically fed with food consisting of 58% maize flour and 42% maize grain (‘overfeeding feed’). The digestive contents from three ducks of each treatment and each genotype were removed at 13 weeks (meal 12) and at 14 weeks (meal 24), 3 h after feeding. Furthermore, at 12 weeks, seven ducks (called not-overfed) were fed normally with 4-mm granules on a restricted diet providing 11.72 MJ kg\(^{-1}\) of food and CP 15.5%. Finally, the animals were killed at 13 and 14 weeks (the same age as overfed ducks).

Sampling for microbiota analysis

The ducks were killed by exsanguination after electric stunning, 3 h following the last meal to homogenize the filling level of the ducks’ digestive tract. Ducks of each genotype with similar weight were selected at three times: at 12 weeks (before overfeeding), 13 weeks (meal 12), and 14 weeks (meal 24). Ileum and ceca were immediately collected and kept on ice. The digestive contents of the ileum and ceca were collected by gently squeezing the organ. The digestive contents of each animal were studied individually and were stored at −20 °C for short-time molecular analysis.

DNA extraction and high-throughput sequencing and analysis of 16S rRNA gene amplicons

Total DNA from ileal and cecal samples was extracted using the QIAamp DNA stool minikit (Qiagen GmbH, Hilden, Germany) according to the instructions of the manufacturer with 220 mg as starting material. An additional lysis step is made using lysozyme (Sigma, Saint-Louis, MO) to improve the DNA extraction of the gram-positive bacteria present within the samples (Johansen et al., 2007). The extracted DNA (from culture or digestive contents) was eluted in 200 µL of elution buffer and stored at −20 °C until real-time PCR analysis. Amplicons from the V3 to V4 regions of 16S rRNA genes (460 bp on Escherichia coli, GenBank accession number J01695) of day 12 samples were amplified using bacterial forward 343F (TACGGRAGGCAGCAG; Liu et al., 2007) and reverse 784R (TACCAGGTATCTAATCCT; Andersson et al., 2008) primers. Each primer had a barcode sequence of ten nucleotides at the 5’ end, which was unique for each sample. The preparation of amplicons was performed in a total volume of 100 µL containing 1× PCR buffer, 200 µM of each dNTP, 1 U lysis DNA polymerase (MP Biomedicals), 0.5 µM of each primers, and 1–5 ng of DNA template. The amplification program consisted of an initial denaturation step at 94 °C for 2 min; 32 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and elongation at 72 °C for 30 s; and a final extension step at 72 °C for 7 min. The PCR products were purified with the QIAquick PCR Purification kit (Qiagen) followed by DNA yield quantification and quality estimation using a NanoDrop ND-100 spectrophotometer. The size of the PCR products was confirmed by gel electrophoresis, and then, the purified PCR products were quantified using the Quant-iT.
Table 1. Ingredients and chemical composition of the experimental diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Nutrient Level (%) of raw materials</th>
<th>Overfeeding feed (dry corn)</th>
<th>Nutrient Level (%) of raw materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>35</td>
<td>ME Vol kg⁻¹</td>
<td>2085</td>
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<tr>
<td>Corn</td>
<td>38.69</td>
<td>Humidity</td>
<td>12.61</td>
</tr>
<tr>
<td>Rapeseed oil free</td>
<td>12</td>
<td>CP</td>
<td>15</td>
</tr>
<tr>
<td>Sunflower cake</td>
<td>11.35</td>
<td>Fat</td>
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</tr>
<tr>
<td>Sodium carbonate</td>
<td>1.375</td>
<td>Cellulose</td>
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</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.575</td>
<td>Ashes</td>
<td>4.99</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.298</td>
<td>Starch</td>
<td>47.32</td>
</tr>
<tr>
<td>Salt</td>
<td>0.36</td>
<td>Nutrient level (% of raw material)</td>
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<tr>
<td>Methionine</td>
<td>0.086</td>
<td>Lysine</td>
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<td>Choline chloride</td>
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<td>Methionine</td>
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<td>Premix + vitamin</td>
<td>0.23</td>
<td>Meth + cystine</td>
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<td>Threonine</td>
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<td>Linolic Ac</td>
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<td>Calcium %</td>
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<tr>
<td>Phosphorus</td>
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<tr>
<td>Sodium</td>
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<tr>
<td>Available Phosphorus</td>
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<tr>
<td>pH</td>
<td></td>
<td>pH</td>
<td>5.36</td>
</tr>
</tbody>
</table>

CP, crude protein.
*Dry matter %.

PicoGreen dsDNA Assay Kit (Invitrogen, Saint Aubin, France) on a ABI prism 7900 HT sequence detection system (Life technology, Invitrogen, A-BIOSYSTEM, Villebon-sur-Yvette, France). The sequencing of the 16S rRNA genes was performed by 454 GS FLX (454 Life Sciences – Roche) and Titanium chemistry according to the manufacturer’s instructions. Amplicons of 450 bp were pooled using equal amounts of each PCR product. For the analysis, samples were recognized by couples of tag sequence, which had been fixed on the universal primers provided by Roche during the amplification.

**Pyrotag handling and analysis**

A total of 498 772 16S rRNA gene sequences (also referred to as 16S pyrotags) were obtained from 454 Titanium pyrosequencing run for the 49 samples. The 16S pyrotags were sorted on the basis of their respective barcodes to form a total of 49 pyrotag library representing the 49 collected ileal and cecal samples. Sequences were sequentially filtered using a Python script developed by the bioinformatic platform of Toulouse, first removing those sequences with a short sequencing length (< 150 nt; 56 393 sequences removed), those with at least one ambiguous base (13 759 sequences removed) or with a long homopolymer (> 8; 206 sequences), those which did not match the proximal PCR primer sequences (with two mismatches allowed; 1441 sequences removed), and finally those having both primers, but with a length shorter than 350 pb (912 sequences removed). A total of 426 061 sequences were retained corresponding to 9582 ± 5624 sequences per samples.

**Taxonomical classification and statistical analysis**

Filtered sequences were analyzed using MOTHUR software, version 1.24 (Schloss et al., 2009). Readings were aligned over the SILVA alignment database provided by MOTHUR software (14 956 sequences corresponding to the unique sequences in the SSU REF database v102; Pruesse et al., 2007), and an alignment quality was calculated using the SILVA secondary structure map file (1072 sequences were removed). After calculating a pairwise distance between aligned sequences, they were clustered into operational taxonomic units (OTU, cutoff of 0.05 using a furthest neighbor clustering). Rarefaction curves, abundance-based coverage estimator (ACE), and Chao1 richness were calculated including rarefaction and Chao1 estimator. The Shannon diversity index was calculated according to Hayek & Buzas (1996). The sequences were analyzed using R (http://www.r-project.org/) to obtain the results of the composition of the microbiota in our treatment conditions. Statistical analysis of similarity (ANOSIM) between band patterns was carried out using 10 000 permutations. The ANOSIM R-value indicated the extent to which the groups differed (R > 0.75, well-separated groups; 0.50 < R < 0.75, separated but overlapping
groups; and 0.25 < R < 0.50, separated but strongly overlapping groups). These proximity values were also graphically explored by nonmetric multidimensional scaling (nMDS). When values are given in the text, they are expressed as mean ± SEM.

Results

Ileal microbial community in Pekin and Muscovy ducks

The microbial diversity in ileal samples of Pekin and Muscovy ducks was estimated by calculating the number of OTUs. In Pekin ducks, the number of OTUs with a cutoff of 0.05 was 1147 ± 638 with coverage per sample of 91.1 ± 3.3%. The average number of sequences was 5202 ± 3435, and 12 different phyla were listed for 184 different taxa. Furthermore, the Chao1, the ACE, and the Shannon index were 2167 ± 747, and 4.0 ± 0.5, respectively (Table 2). In Muscovy ducks, 1022 ± 275 OTUs with a cutoff of 0.05 were detected. The average number of sequences per sample was 11 533 ± 1944, and the sequences were affiliated with nine phyla and 214 different taxa. The different diversity indices for these samples before overfeeding were 1816 ± 384 for the Chao1 indices, 2420 ± 510 for ACE, and 5.2 ± 0.8 for the Shannon diversity index (Table 2). The two major phyla in both Pekin and Muscovy ducks were \textit{Firmicutes} (71.5 ± 13.2% and 48.7 ± 11.9%, respectively) and \textit{Bacteroidetes} (16.4 ± 15.3% and 21.8 ± 12.3%, respectively). \textit{Proteobacteria} represented around 10.6 ± 6.0% in Pekin and 26.4 ± 9.6% in Muscovy (Fig. 1a). Finally, other phyla such as \textit{Actinobacteria}, \textit{Fusobacteria}, \textit{Deferribacteres}, \textit{Spirochetes}, or \textit{Acidobacteria} represented < 2% or 3.5% of the population in Pekin and Muscovy ducks, respectively (Fig. 1a). To evaluate the microbiota composition at finer taxonomic levels, class distributions were analyzed. In Pekin ducks, \textit{Firmicutes} were dominated by \textit{Bacilli} (53.6%) and \textit{Clostridia} (22.9%), \textit{Bacteroidia} (from the phylum \textit{Bacteroidetes}) accounted for 16.1%, and the classes \textit{Gammaproteobacteria} and \textit{Deltaproteobacteria} that are part of the \textit{Proteobacteria} were represented at levels of 3.9% and 0.4%, respectively (Fig. 1b). The \textit{Actinobacteria} represented < 1% of the population of the ileum (Fig. 1b). In Muscovy ducks, \textit{Firmicutes} were mainly composed of \textit{Clostridia} (36.7%), \textit{Bacilli} (11.7%), and \textit{Bacteroidia} (21%). Regarding the phylum \textit{Proteobacteria}, the major classes were \textit{Gammaproteobacteria} (18.1%) and \textit{Deltaproteobacteria} (5.6%) (Fig. 1b). Finally, 173 genera were detected in Pekin ducks, and 196 genera, in Muscovy ducks.

Cecal microbial community in Pekin and Muscovy ducks

The average number of ceca OTUs with a cutoff of 0.05 was 901 ± 137 with coverage per sample of 91.1 ± 3.3% in Pekin ducks, whereas there were 1251 ± 569 OTUs in Muscovy ducks (Table 2). The average number of sequences in Pekin was 8626 ± 2614 with eight phyla represented by 111 different taxa and in Muscovy 8784 ± 5979 represented by 118 taxa grouped in eight different phyla. Regarding the Chao1, the ACE, and the Shannon index, the values were 1782 ± 390, 2631 ± 493, and 5.2 ± 0.2, respectively, in Pekin ducks and 2512 ± 469, 3765 ± 706, and 5.8 ± 0.2, respectively, in Muscovy ducks. In Pekin ducks, the cecal microbiota was mainly composed of \textit{Bacteroidetes} (64.8 ± 2.5%) and \textit{Firmicutes} representing 27.5 ± 3.3% of the population, while there was only 3.92 ± 1.2% of \textit{Proteobacteria}. In Muscovy ducks, \textit{Bacteroidetes} (50.9 ± 2.1%), \textit{Firmicutes} (40.5 ± 2.9%), and \textit{Proteobacteria} (7.1 ± 1.3%) were also dominant. Other phyla, such as \textit{Actinobacteria}, \textit{Deferribacteres}, \textit{Spirochetes}, and \textit{Synergistetes}, represented < 2% of

<table>
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<tr>
<th>Segment Period</th>
<th>Ileum</th>
<th>Ceca</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Bof</td>
<td>Mof</td>
</tr>
<tr>
<td>Muscovy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of operational taxonomic unit</td>
<td>1022</td>
<td>345</td>
</tr>
<tr>
<td>Chao1 estimated richness</td>
<td>1816</td>
<td>756</td>
</tr>
<tr>
<td>ACE</td>
<td>2420</td>
<td>807</td>
</tr>
<tr>
<td>Shannon diversity index</td>
<td>5.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Pekin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of operational taxonomic unit</td>
<td>1147</td>
<td>480</td>
</tr>
<tr>
<td>Chao1 estimated richness</td>
<td>2167</td>
<td>951</td>
</tr>
<tr>
<td>ACE</td>
<td>3114</td>
<td>1313</td>
</tr>
<tr>
<td>Shannon diversity index</td>
<td>4.0</td>
<td>2.9</td>
</tr>
</tbody>
</table>

ACE, abundance-based coverage estimator; Bof, before overfeeding (12 weeks); Mof, mid-overfeeding (13 weeks); Eof, end overfeeding. *Standard error of the mean.
the community and were not present in all samples (Fig. 2a). At the class level, the Bacteroidetes was dominated by Bacteroidia (64.3% in Pekin and 50.1% in Muscovy) and Clostridia (23.1% in Pekin and 37.1%). Bacilli sequences accounted for 4.2% and 2.6% from the Firmicutes phyla and those from Deltaproteobacteria for 1.4% and 5.8%, in Pekin and Muscovy duck samples, respectively. Gammaproteobacteria (1.2%) and Actinobacteria were detected only in Pekin duck samples (Fig. 2b). Sequences from the other phyla accounted for < 2% of the community (Fig. 2a). Finally, in Pekin and Muscovy cecal samples, 88 and 113 genera were detected, respectively.

**Effect of overfeeding on the ileal and cecal microbial community in Pekin and Muscovy ducks**

Overfeeding reduced Chao1 richness, the ACE, and the Shannon index in ileal samples, whereas these different richness or diversity estimators increased in cecal samples regardless of the genotype of ducks, but with variation (Table 2).

**Effect on ileum**

Furthermore, the ratio Firmicutes/Bacteroidetes in ileal samples tended to increase during overfeeding, but with high variation (Bof = 43.8 ± 20.7, Mof = 3082.5 ±1589.5, Eof = 507.6 ± 444.7) in Pekin and (Bof = 156.1 ± 92.1, Mof = 471.8 ± 337.2, Eof = 408.7 ± 321.9) in Muscovy ducks (Fig. 7). After the different level of intake (overfeeding or not) and the length of the overfeeding period (1 or 2 weeks), the comparison of bacterial communities was analyzed by nMDS profiles (representing the distribution of different families). Ileal sampling distinguished two separate clusters: overfed ducks were found in one cluster and the not-overfed ones in others regardless of the length of overfeeding or genotype of the animals (Fig. 3). The ANOSIM R-value indicated the extent to which the
groups differed. For ileal samples, two separate but overlapping groups were observed: $R_{ANOSIM} = 0.575$ and a $P < 0.001$, which indicates an effect of overfeeding on the bacterial community. Furthermore, there was also an effect of the overfeeding period, namely a statistical difference between the first period (at 12 weeks or before overfeeding) and the last one (at 14 weeks). Two separated but strongly overlapping groups were obtained with $R_{ANOSIM} = 0.419$ and a $P = 0.009$. This statistical difference was only observable between these two periods (data not shown). The separation of samples by the level of intake was confirmed using the heat map method (Fig. 4). The different effects observed were due to changes in different families such as *Leuconostocaceae* for the genotype effect or mainly *Lactobacillaceae* or *Streptococcaceae* for the period effect. All statistical changes ($P$-value $< 0.05$) are listed in Table 3, and there were no interactions. For each sample, the diversity of all families detected has been observed; families with the largest diversity were *Clostridiaceae*, *Lactobacillaceae*, *Ruminococcaceae*, and

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**Fig. 2.** Percentage distribution of sequences (%) evaluated at the phylum (a) and class (b) levels to the total number of sequences in the ceca of Muscovy (M) and Pekin (P) ducks during overfeeding (Bof, before overfeeding (12 weeks); Mof, mid-overfeeding (13 weeks); Eof, end of overfeeding).

**Fig. 3.** nMDS profile showing the distribution of different families of the different samples studied overfed (●) or not-overfed (△) in three periods of overfeeding in the ileum. Before overfeeding (1), mid-overfeeding (2), and end (3) of overfeeding.
Fig. 4. Heat map of the bacterial community at the family levels of Muscovy (M) and Pekin (P) for overfed (OF) or not-overfed (NOF) ducks in the ileum. Samples and taxa were clustered according to Ward algorithm based on a Manhattan distance matrix. Before overfeeding (1), mid-overfeeding (2), and end (3) of overfeeding.
Streptococcaceae, which played an important role in the clustering (Fig. 4).

**Effect on ceca**

In ceca samples, the ratio Firmicutes/Bacteroidetes (Bof = 0.4 ± 0.1, Mof = 0.3 ± 0.1, Eof = 0.3 ± 0.1) was stable in Pekin ducks during overfeeding, whereas the richness estimators increased. Regarding cecal samples, nMDS profiles also showed that the samples were slightly separated according to the treatment (overfed or not): R\textsubscript{ANOSIM} = 0.116 and \( P = 0.010 \) (Fig. 5a). As in ileal samples, the heat map method revealed the separation by genotype and level of intake (Fig. 6). The Firmicutes/Bacteroidetes ratio in Muscovy ducks tended to decrease at mid-overfeeding and increase at the end of overfeeding (Bof = 0.8 ± 0.1, Mof = 0.5 ± 0.1, Eof = 0.9 ± 0.6; Fig. 7). A significant difference (\( P < 0.05 \)) was observed between the two genotypes for the Firmicutes/Bacteroidetes ratio. Several families were responsible for these effects especially Bacteroidaceae, Desulfovibrionaceae, and Lachnospiraceae for the genotype effect or Streptococcaceae for the overfeeding effect. All changes for these families were significant (\( P < 0.05 \)) and are listed in Table 4. In our samples, seven families showed a high diversity: Lachnospiraceae, Porphyromonadaceae, Rikenellaceae, Prevotellaceae, Ruminococcaceae, and Bacteroidaceae. Interestingly, there was also a genotype effect between Pekin and Muscovy ducks regardless of age or the period of overfeeding, with a few separations in to groups: R\textsubscript{ANOSIM} = 0.195 and \( P = 0.001 \) (Figs 5b and 6).

**Discussion**

In this study, high-throughput sequencing was used for the first time to identify the microbial diversity of ileal and cecal samples from ducks, after which the effect of overfeeding or genetics on that diversity was studied. At the phylum level, Firmicutes and Bacteroidetes (more than 80% of sequences) were major bacteria in the duck gut microbiota. Most studies on microbiota have shown that Firmicutes and Bacteroidetes are the two most dominant phyla in different organisms such as birds or mammals suggesting an important conserved role in intestinal microbiota metabolism (Ley et al., 2008; Kohl, 2012). Costello et al. (2010) suggest that the common ancestor of amniotes (birds, reptiles, and mammals) harbored a
Fig. 6. Heat map of the bacterial community at the family levels of Muscovy (M) and Pekin (P) for overfed (OF) or not-overfed (NOF) ducks in the ceca. Samples and taxa were clustered according to a Ward algorithm based on a Manhattan distance matrix. Before overfeeding (1), mid-overfeeding (2), and end (3) of overfeeding.
microbiota composed essentially of *Firmicutes* and *Bacteroidetes*. Furthermore, as previously described in chickens, ceca were dominated by obligate anaerobes (*Bacteroidetes* including *Bacteroidia* and *Firmicutes* including *Clostridia*) in both Pekin and Muscovy ducks, but differences occur with chickens. In this study, cecal segments were dominated by *Bacteroidetes* in ducks, whereas *Firmicutes* (especially the class *Clostridia*) is the major phylum in chickens (Zhu et al., 2002; Lu et al., 2003). Interestingly, turkeys also show *Bacteroidetes* as the major phylum in cecum regardless of the phylogenetic distance between turkey and ducks, suggesting that environmental conditions and diet could play a more important role than genetics. Furthermore, although *Firmicutes* were dominant in ileal segments in chickens and ducks, interesting changes have been identified at the class and family levels (Barnes, 1979; Mead, 1989; Lu et al., 2003). In chickens and Pekin ducks, sequences related to the *Bacilli* order (from *Firmicutes*) were dominant (80% for chickens and 51% for ducks), but the family distribution was quite different. In Pekin ducks, the genera *Streptococcus* spp. and *Enterococcus* spp. represented most of the *Bacilli* sequences (50%), while in chickens, around 70% of sequences are related to the *Lactobacillus* genus (Lu et al., 2008). Regarding Muscovy ducks, the bacterial community in the ileum was not dominated by facultative anaerobes, but by obligate anaerobes such as *Clostridia* and *Bacteroidia*. In humans, the ratio *Firmicutes*/*Bacteroidetes* is closely related to weight; it increases in obese people and may decrease during weight loss (Ley et al., 2006). In this study, it tended to increase in the ileal segment, but with high variation (Fig. 7). The *Firmicutes*/*Bacteroidetes* ratio appeared to be correlated with overfeeding in the ileum and with genotype in the ceca. Overfeeding in ducks promotes an important hepatic steatosis induced by an increased rate of lipogenesis from carbohydrates and a defect in hepatic lipid secretion (Sadowski & Leclercq, 1987; Fournier et al., 1997). It is well known that overfed Pekin ducks have a higher lipid content in peripheral tissues (muscle and adipose tissues), while liver storage is lower than in other species (Davail et al., 2003a; Chartin et al., 2006). Otherwise, the LPL activity in ducks correlates positively with a higher storage in peripheral tissues instead of fat storage in the liver (Saëz et al., 2010). Interestingly, in mammals, the microbiota triggers the storage of triglycerides through suppression of the intestinal expression of fasting-induced adipocyte factor, a circulating LPL inhibitor (Backhed et al., 2004). Could these changes in the microbial community during overfeeding affect a predisposition to liver or peripheral fat storage in ducks? In our study, overfeeding reduced the bacterial diversity estimated by the Shannon index in ileal samples. Overfeeding affected the relative abundance of *Firmicutes* and especially some genera from the *Bacilli* class (*Lactobacillaceae*, *Enterococcaceae*, and *Streptococcaae*) regardless the genotype. The relative abundance of *Lactobacillaceae* spp. increased strongly with overfeeding in both Pekin and Muscovy ducks leading to a decrease in most other families. These bacteria are well known as amylolytic and lactate-producing bacteria and frequently increase in animals (e.g. pigs and rats) fed with diets rich in starch (Wang et al., 2002; Regmi et al., 2011). In cattle, it has been shown that *Lactobacillus* spp. increase with high-concentrate diets containing more than 70% of starch (Brown et al., 2006). Furthermore, *Streptococcaceae*, another amylolytic bacterial family, is decreased by overfeeding in Pekin and Muscovy ducks, whereas in cows, Fernando et al. (2010) report the increase in *Streptococcus* spp. with a high grain diet and especially *Streptococcus bovis*. In birds, the effect of diets on the abundance of *Enterococcaceae* spp. and *Streptococcus* spp. has not yet been published. Another member of *Bacilli*, the *Enterococcaceae* family, was affected, but only in Pekin ducks. Very little is known in birds regarding the effect of diets on these different families. A very small effect of the genotype was also detected in the *Leuconostocaceae* family, from the class *Bacilli*, a new family identified in 2010 (Schleifer, 2010). Finally, regarding ileal samples, regardless of the genotype and the length of the diet, they were only separated by the diet type (overfed or not), and the most affected bacterial groups were the *Lactobacillaceae*, *Lachnospiraceae*, *Bacteroidaceae*, *Streptococcaceae*, and *Ruminococcaceae* (Fig. 4 or Fig. 3). Interestingly, in cecal samples, the effect of the genotype was more

Table 4. Statistical effect of the genotype and period on different families with a significant *P*-value (*P < 0.05) in the ceca of Muscovy and Pekin ducks

<table>
<thead>
<tr>
<th>Genotype effect</th>
<th>Muscovy</th>
<th>Pekin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P</em>-value</td>
<td>Mean</td>
</tr>
<tr>
<td><em>Deferribacteraceae</em></td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td><em>Succinivibrionaceae</em></td>
<td>0.003</td>
<td>0.07</td>
</tr>
<tr>
<td><em>Peptococcaceae</em></td>
<td>0.005</td>
<td>9.67</td>
</tr>
<tr>
<td><em>Lachnospiraceae</em></td>
<td>0.006</td>
<td>0.30</td>
</tr>
<tr>
<td><em>Clostridiaceae</em></td>
<td>0.008</td>
<td>0.08</td>
</tr>
<tr>
<td><em>Bacteroidaceae</em></td>
<td>0.01</td>
<td>25.12</td>
</tr>
<tr>
<td><em>Desulfovibrionaceae</em></td>
<td>0.019</td>
<td>8.94</td>
</tr>
<tr>
<td><em>Fusobacteriaceae</em></td>
<td>0.037</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Feeding period effect

| Comamonadaceae       | 0.001 | 0.02 | 0.01 | 0.02 | 0.01 |
| Streptococcaceae     | 0.004 | 1.85 | 0.78 | 0.57 | 0.28 |
| Anaeroplasmataceae   | 0.023 | 0.03 | 0.01 | 0.05 | 0.02 |

*Standard error of the mean.
important than the effect of overfeeding, suggesting that
the complexity and the anaerobic environment of the ceca
did not allow important changes in the bacterial community.
Furthermore, it has been suggested that transit times
are longer in the ceca, permitting a better microbial fermenta-
tion (Rehman et al., 2007). The physiology of Pekin
and Muscovy ducks is quite different as previously
described, and several metabolites such as short-chain fatty
acids (SCFA) or amino acids from the ceca could be a part
of that difference (Józefiak et al., 2004). Three families
show significant differences between Pekin and Muscovy
ducks: Lachnospiraceae, Bacteroidaceae, and Desulfovibrion-
aceae from three different phyla. Moreover, Lachnospira-
ceae from Firmicutes and Bacteroidaceae are two families
with bacteria with amylolytic and cellulolytic properties
(Gotta, 1987; Wedekind et al., 1988; Flint et al., 2012).

Regarding Desulfovibrionaceae, some family members can
produce acetate (one well known SCFA) and are among
the sulfate-reducing bacteria (Devereux et al., 1990). There
was also a need to find an effect of overfeeding on three
families in both Pekin and Muscovy ducks: Streptococcaceae
in ileal samples and two poorly documented families,
Comamonadaceae (from Proteobacteria) and Coriobacteri-
ales (wall-less bacteria belonging to Firmicutes and the Mol-
licutes class). Finally, in cecal samples, the bacterial
community could be separated by both the genotype
(Fig. 5a) and the level of intake (Fig. 5b), but the former
had the greater effect. Heat maps confirmed these results,
and the most affected bacterial groups were the families
described above (Fig. 6). The genetic effect on microbiota
composition was also well described in mice (Friswell
et al., 2010; Kovacs et al., 2010).
In conclusion, this study using a high-throughput pyrosequencing based on the 16S rRNA gene in ileal and cecal samples from ducks provides a first inventory of the microbial community and the effect of overfeeding and genotype on the abundance of major different groups. Overfeeding affected the richness diversity of ileal and cecal samples and had a significant effect in modifying the bacterial community in the ileum, whereas genotype mainly affected the ceca. The microbial diversity of ducks’ microbiota was dominated by Firmicutes and Bacteroidetes. Further investigations are necessary to understand the functional microbiota in ducks and enhance the digestibility of resistant starch during overfeeding periods in ducks. This would be of great interest for researchers studying intestinal microbiota in poultry, and this new knowledge could be used to prepare birds to overfeeding.

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