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BREEDING FOR SUCCESS … TOGETHER

LOHMANN TIERZUCHT

Contents

Dear colleagues and friends,

The role of the microbiota in humans and animals has been underestimated in the past. In animal science the microbiota of the intestinal tract was mainly studied with regard to the digestion of fibre in ruminants. In chickens and pigs as monogastric animals dietary fibre was considered as bulk which hampered the formulation of high density rations, or, caused problems when presented as non-starch polysaccharides (NSP). Veterinary studies mainly focused on pathogenic microbial organisms. There is increasing awareness that the microbiota has important role to play beyond digestion and infections. It has been shown that metabolites of microbial origin act as neurotransmitters and influence the regulation of feed intake and mental state of humans and animals. Differences in the composition of the microbiota in laying hens of high and low feather pecking activity suggest an influence of the microbial activity on this damaging behaviour. Recent results have shown that the microbiota of the digestive tract influence the immune response of animals and, thus susceptibility to diseases. Conventional microbiological studies of the complex composition of the microbiota in the digestive tract, in feed or on carcasses were extremely difficult and time consuming. Recently developed methods of molecular genetics allow the analysis of large number of samples within short time qualitatively as well as quantitatively. Concomitantly with the development of new techniques, new technical terms have emerged from this new field of research. Alessandra de Cesare contributes an introduction of the different methods and related terminology. She also presents examples of application of metabolomics and an outlook of the importance of these studies on poultry nutrition, health and product quality.

Breast muscle myopathies cause economic losses in the broiler industry and are considered to compromise the welfare of affected birds. Massimiliano Petracci and his working group have been working extensively on this problem. Results of research from the literature and their own laboratory are reviewed in the second article, and possibilities to minimize the problem are discussed.

Food safety is the most important issue for consumers when purchasing meat and meat products. Reports of the public media on this subject are often focused on scandals and provide the impression that the consumption of poultry products in particular is extremely dangerous and threatens our health. These alarm calls are not always based on sound scientific studies (see Konrad Biesalski https://www.ltz.de/en/news/lohmann-information/Meat-as-a-healthy-and-valuable-source-of-micronutrients.php). It is therefore important to regularly inform producers, retailers and consumers on the scientific state of health risks emerging form poultry products. In the third article Hafez M. Hafez presents the latest state of knowledge on this topic.

The duck is a poultry species with high potential for meat and egg production. Research on breeding and production of commercial ducks is limited, especially in Europe and North America, but genetic selection for improved efficiency and meat quality has been practiced successfully and continues. Information on the production level reported in most textbooks is outdated. The Bavarian Center for Small Animal Research and Extension, Kitzingen, Germany, recently summarized up-to-date data on the productivity of commercial duck breeds, which are commonly used in Germany and neighboring countries. The study of Christian Wild and co-authors is not based on a random sample test, but the means and ranges presented for different economic criteria are typical for the breeds under field conditions. The results show rapid growth rate and high yield. The variation among breeds indicates scope for future development.

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Metagenomics to investigate the dynamics of microbial communities in poultry and poultry products

Abstract

The poultry gut microbiome represents gut microorganisms and genomes as well as genes belonging to those microorganisms. Since the gut microbiome is involved in the regulation of multiple host metabolic pathways, a deep understanding of the relationships between gut microbiome and host should provide new strategies to improve poultry health and productivity as well as poultry meat safety. In this review, metagenomics techniques currently available are summarised, as well as possible approaches to data analysis. Furthermore, selected metagenomic studies illustrating how to implement metagenomic projects to find the reasons of changes in chicken productive performances due to nutritional interventions, to map variations in antibiotic resistance genes and to investigate the microflora associated to poultry carcasses are shown, along with key technical issues to address in order to promote metagenomics investigations in poultry science.

Keywords

Metagenomics, next generation sequencing, microbiota, antibiotic resistant genes, meat safety.

Introduction

The number of microbial cells and genes in chickens overcome the total of poultry cells. Since the great majority of microbes live in the chicken gut, to understand and exploit the impact of microbes on physiological processes of chickens, content, diversity and functioning of the microbial gut community must be decoded. The collective microbial community inhabiting the chicken gut is named microbiota, whereas the collective genomic content

of the microbiota is referred as metagenome. Furthermore, the ecosystem represented by all the microorganisms (i.e. bacteria, archaea, lower and higher eukaryotes, and viruses) inhabiting the chicken gut, their genes as well as the chicken genes, is defined microbiome (Tremaroli and Bäckhed, 2012). The next generation sequencing (NGS) approaches to characterize the microbiota, the metagenome and the microbiome are different one to the other and details on the available sequencing strategies are provided below.

Metataxonomic

The sequencing-based approach to identify the entire microbiota in a sample is named metataxonomic and refers to sequencing of marker genes followed by assignment of each obtained sequence to a taxonomic level, from phylum to species or even subspecies, using a variety of bioinformatic tools. One of the most general targets for metataxonomic is the hypervariable region in genes encoding the 16S rRNA. The bacterial 16S rRNA gene possesses nine hypervariable regions (V1-V9) **(Figure 1).**

Sequence variations in hypervariable regions allow accurate bacterial taxonomic estimation by comparing against 16S rRNA gene sequences deposited in public databases, such as GreenGenes, Ribosomal Database Project, SILVA, etc. (Choi et al., 2015). The overall sequencing output of the 16S rRNA is a cluster of nearly identical sequences, referred to operational taxonomic units (OTUs) (Cole et al., 2014). The representative sequences, which are either the most abundant sequences or sequence with the least distance summation within all sequences in the same OTUs, are then matched to those in a public database to obtain taxonomic classification. In addition to providing taxonomic information, the OTUs provide information on population diversity, indicating richness and evenness of individual species in a sample as alpha diversity (Colwell, 2009). This information is also used to account for the degree of divergence between different communities or sample types.

Metagenomic

The sequencing based approach to characterize both the metagenome and the microbiome does not target a specific gene, like the 16S rRNA but is shotgun, meaning random on the whole genomic content. Shotgun sequencing is the process of randomly breaking (often by shearing) long DNA molecules (for example, complete chromosomes) and then sequencing the resultant DNA fragments, which each come from a different location in the original DNA molecule (Weinstock, 2012). Shotgun sequence data provide information on the organisms that make up communities but also information on functional genes in the sample. Therefore, the current challenge for metagenomic sequencing is to complete the framework associating each specific microorganism with its own gene set.

Besides technical differences between metataxonomic and metagenomic, the goals for any metagenomic project are to understand: 1) community composition/structure, including taxonomic breakdown and relative abundance of the various species; 2) genic contribution of each member of the community, including number and functional capacity; 3) intra-species and/or intra-population gene heterogeneity (Scholz et al., 2012).

Next Generation Sequencing (NGS)

Both metataxonomic and metagenomic have been made possible by the advent of (NGS technologies, also defined as massively parallel sequencing because they allow for millions of sequencing reactions to happen in parallel. NGS technologies can be divided into Second Generation Sequencing (SGS) and Third Generation Sequencing TGS), based on the sequencing process used (Ambardar et al., 2016).

The combination of read (i.e., sequence) length and number of reads defines the throughput of each instrument in number of bases per run. When the human genome project started in 1990 using the Sanger sequencing, which is not a massive sequencing strategy, 13 years and 2.7 billion US dollars have been spent to sequence one single human genome, which is about 3 billion base pair, meaning 3 times the chicken genome. Today, by using the second and third generation of sequencing technologies, 100000 human genomes have been sequenced and each one costs less than 1000 US dollars. Nevertheless, the bottleneck is now computational and data storage as well as data interpretation.

Fig 1. Hypervariable regions (V1-V9) in the 16S rRNA

[1] *Based on an invited paper, presented at the EggMeat Symposium of the WPSA 2017 in Edinburgh*

Table 1. Overall description of the steps included in a metagenomic study

Wet lab part
Sample collection, transport and storage
Sample handling
DNA or RNA extraction and quantification
Library preparation and quantification
Sequencing
Dry Lab part
Quality control of the sequences
Trimming of indexes and adaptors
Bioinformatics analysis of the sequences
Biostatistic analysis of differences between sequencing results of different samples

Data analysis strategies

The simplest way to analyse the metagenome is to compare the obtained reads (i.e., sequences) to sequence databases and calculate the statistical significance of matches to identify taxonomical and functional entities (e.g. https://blast.ncbi.nlm.nih.gov). Alternatively, assembly algorithms can be used to reconstruct short reads into a sequence contig((set of DNA fragments with known sequence), which is a set of overlapping sequences representing the contiguous DNA fragment (Mende et al., 2012; Thomas et al., 2012).

Although obtaining a complete individual genome from metagenomic sequences is still challenging, the data collected are sufficient to characterize the major functions of the microbial communities as well as to identify their taxon, by assigning to public genome reference databases (Howe et al., 2014; Nielsen et al., 2014). Even though current computational analysis strategies for metagenomic data rely largely on comparisons to reference genomes, they represent only a fraction of what we know and therefore limit our ability to segregate metagenomic data into coherent biological entities and fail to describe previously unknown species, phages and modules of genetic variation within microbial species (Nielsen et al., 2014).

A possible alternative is the de novo assembly (i.e., assembly without a reference) of genomes from complex metagenomic data, although it is inherently difficult due to the many sequence ambiguities that confuse the assembly process. Hence, a typical metagenomic assembly will result in a large set of independent contigs that are not easily aggregated into biological entities. A good example of identification and assembly of genomes in complex metagenomics samples without using reference genomes has been described by Nielsen et al. (2014). They clustered the metagenomic data into groups of genes with similar abundance named CAGs. Since the genomes of bacteria contain more than 700 genes, the CAGs with more than 700 genes are considered metagenomic species, whereas the CAGs with less than 700 genes are identified as genes for protein of phages, CRISP associated genes relevant in the immune system against foreign nucleic acids, restriction endonucleases, glycotransferases, etc.

How many samples should I include in a metagenomics study and what should be the sequencing depth?

Each metagenomic study includes different steps grouped in a wet lab part and in a dry lab part (Table1).

Due to the lack of standardization in the wet lab part clear answers on how metagenomic studies should be performed are not available in the literature. However, evidences in the literature can address specific issues like how many samples should be tested in a metagenomic project. Nielsen et al. (2014) sequenced 396 human stool samples and included among the 396 individuals tested 19 people who consumed a fermented milk containing Bifidobacterium animalis sub lactis. This strain was then used by the authors as benchmark to assess the suitability of their method to identify the species. Even though only 0.3% of the sequence reads (DNA fractures with known sequence) in the 19 selected samples originated from B. animalis, 95% of the B. animalis reference genes were captured into one specific metagenomic species. At a sequencing depth of 700000 bp and even 200000 bp, 97% of the B. animalis genes are captured; however at least 18 samples are needed to achieve the same level of specificity meaning that the number of samples to test is more critical than sequencing depth.

Concerning the sequencing depth, Ni et al. (2013) state that the genome of a single species can be accurately assembled from a complex metagenomic dataset when it shows at least 20-fold coverage, meaning that there are at least 20 fold sequence data covering that specific genome. According to their calculation at least 7Gbp (i.e., 7x109 bases) of sequencing output is required to enumerate the gene contents of prokaryotes with relative abundance of

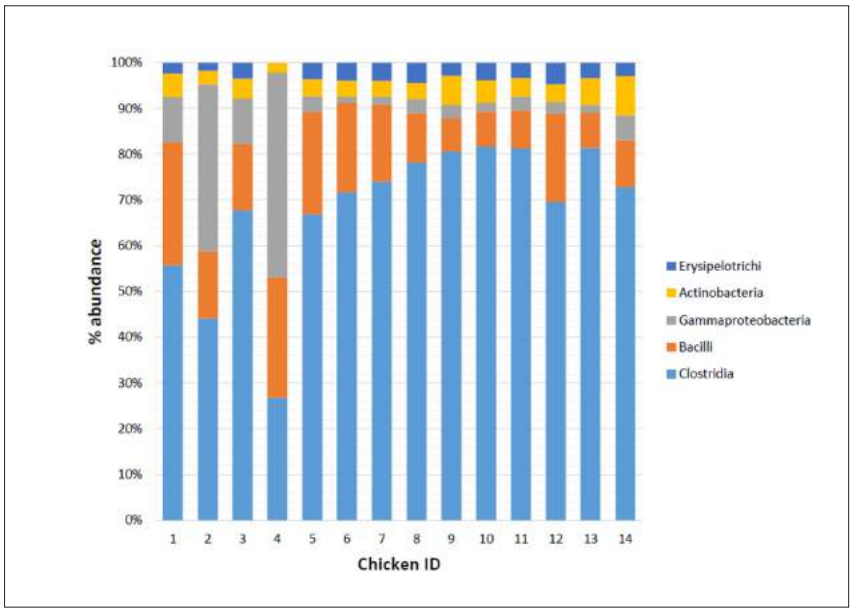

Fig 2. Mean relative frequency of abundance (% abundance) of most represented bacterial classes in caeca contents of chickens belonging to the same trial and tested at Day 1 (chicken IDs 1-4) and 41 in a control (chicken IDs 5-9) and a treated (chicken IDs 10-14) group.

more than 1% in a microbiome. This does not mean that in all the experiments this depth of sequencing must be used but lower depth would map species and genes with relative abundance higher than 1%. In terms of costs, to sequence each sample at 7Gbp means to spend something between 270 and 300 Euro/sample. To include 18 samples/test in a metagenomic poultry study means a lot of money. Besides, birds belonging to the same group, either control or treated one, and reared within the same farm might be considered less prone to variability in comparison to humans as addressed in the study by Nielsen et al. (2014). Therefore, 9 samples/treatment might be considered enough to test. However, that sample number should be really decided case by case according to the metagenomic project goals. As an example **Figure 2** shows that the bacteria classes identified in the caeca contents of chickens tested at day 1 (IDs 1 to 4) and at the end of the rearing period (i.e., 41 days) within both a control group (IDs 5 to 9) and a treated group (IDs 10 to 14) and display differences in their mean relative frequency of abundance in individual chickens. Therefore, decreasing too much the number of samples to investigate in a trial might negatively impact on the detection of the variability between samples.

Metagenomic sequencing to interpret changes in chicken productive performances due to nutritional interventions

Metagenomic studies can help to interpret the reasons of changes in chicken productive performances associated to nutritional interventions. De Cesare et al. (2017a) observed a significant beneficial effect of the dietary supplementation with Lactobacillus acidophilus D2/CSL (CECT 4529) at the recommended dietary dosage of 1x109 cfu/kg feed on (1) broiler body weight gain between 15-28 days; (2) improved feed conversion rate in the overall rearing period (i.e., from 0 to 41 days); (3) a lower incidence of pasty vents in chickens treated with the probiotic. To explain these observations caecum contents were sampled from treated and untreated birds and submitted to metagenomic shotgun sequencing to look at both taxonomic composition as well as functional genes in both control and treated samples. The relative abundance of Lactobacillus acidophilus in the caeca of treated chickens was comparable with that of the control group probably because the colonization preference of the administered strain is the crop and the small intestine. On the other hand, microbial species producing butyric acid, such as Ruminococcus obeum, Clostridium clostridioforme, Roseburia intestinalis, Lachnospiraceae bacterium 14-2T and Coprococcus eutactus at 41 days displayed a significantly higher relative frequency of abundance in the treated birds in comparison to the control group **(Figure 3)**. This result suggests that besides the lack of colonization of Lactobacillus acidophilus in broiler caeca, the metabolic activity of the supplemented probiotic positively affected species producing butyric acid by a cross feeding mechanism.

Concerning the lower incidence of pasty vents in the chickens treated with the probiotic it was associated to lower abundance of Ruminococcus torques in their caeca. Indeed, R. torques is known to degrade the gastrointestinal mucin, representing a carbon and energy source for intestinal microbiome. It has been estimated that 1% of colonic microbiome is able to degrade host mucin using enzymes (e.g. glycosidases and sulfatases) that can degrade the oligosaccharide chains. Moreover, degradation of mucin is regarded as a pathogenicity factor, since loss of the protective mucus layer may expose gastrointestinal tract cells to pathogens. Therefore, the higher abundance of R. torques in the control group was probably related to the higher incidence of pasty vent in the control group in comparison to the treated group.

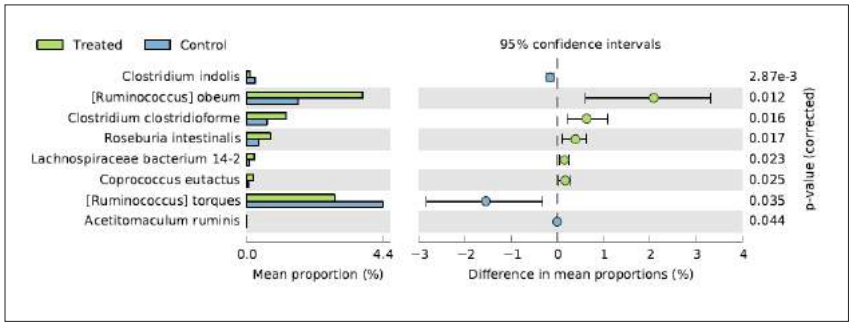

Fig 3. Bacterial species significantly differed in chickens treated with L. acidophilus (Treated) in comparison to the untreated birds (Control) at 41 days.

In relation to the metabolic functions, the caecum contents collected in the treated group showed a significantly higher level of β-glucosidase **(Figure 4)**. This enzyme contributes to the hydrolysis of glucose monomers from non-starch polysaccharides (e.g., cellulose, β-glucans), playing an important role in the fermentation of undigested carbohydrates and, ultimately, in animal performance and health. In particular, β-glucosidase (β-glucoside glucohydrolase; EC3.2.1.21) hydrolyzes alkyl- and aryl-β-glucosides, as well as diglucosides and oligosaccharides, to release glucose and an aglycone. It also hydrolyzes isoflavonal glycoside conjugates into isoflavone aglycones, such as genistein, daidzein, and glycitein. These aglycones hydrolyzed by

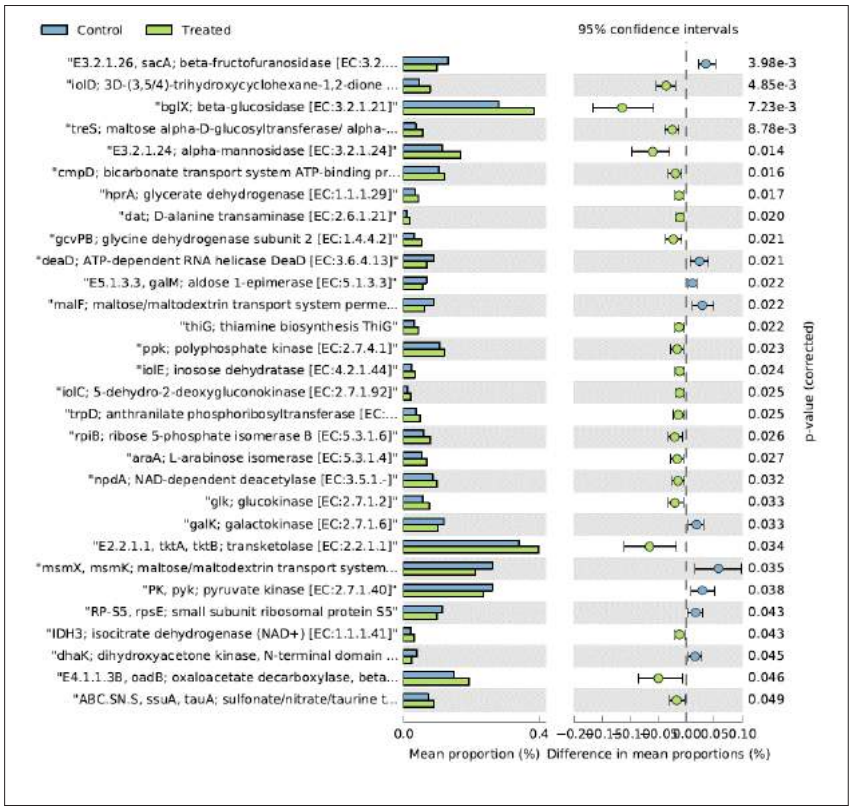

Fig 4. Mean relative frequency of abundance (% abundance) of the KEGG functions showing P < 0.05 between chickens treated with L. acidophilus (Treated) in comparison to the untreated birds (Control) at 41 days.

β-glucosidases from intestinal microorganisms are readily absorbed across the villi of the intestine, possess greater bioavailability than the corresponding glycoside conjugates and a wide range of biological properties, such as antioxidant and anti-tumor activities.

Metagenomic mapping of variations in antibiotic resistance genes

A further application of metagenomics concerns the investigation of antibiotic resistance genes (ARGs) in chicken guts, poultry faeces and farm environments (Xiong et al., 2018). Animal faecal microflora harbours a vast reservoir of ARGs that could be acquired by human commensals and pathogens. Furthermore, antibiotic resistant bacteria and ARGs in animal excretion may be transported into the environment via manure application, leakage, runoff and airborne particulate matter, globally contributing to the aggregation of resistance in the environment. Xiong et al.(2018) applied metagenomic sequencing to investigate variations in ARGs and bacterial host abundance in the faeces of broilers treated for 5 days with a low dose (0.2 g/L) and a therapeutic dose (2 g/L) of chlortetracycline in the drinking water. The results obtained showed that therapeutic dose of chlortetracycline inhibited multidrug resistance genes (i.e., mdtA, mdtC, mdtK, ompR, and TolC) and promoted the abundance of tetracycline resistance genes (tetA and tetW). The metagenomic analysis was performed at 8.7 Gbp of reads for each sample and showed that the resistome is quite similar in all tested groups and is established in the poultry fecal metagenomes also in absence of antibiotic exposure. However, the principal component analysis **(Figure 5)** shows a clear shift in the antibiotic resistance subtypes over time, meaning between T0=27 days of

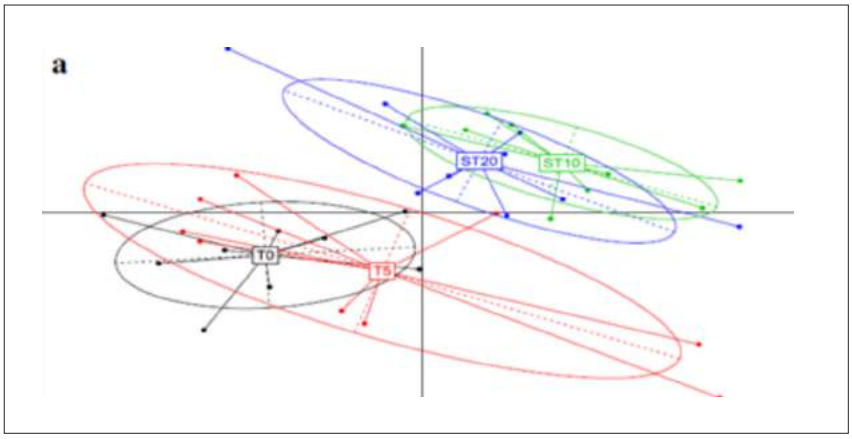

Fig 5. Principal component analysis of ARG subtypes over the course of chlortetracycline administration. T0=27 days of rearing and starting time of the treatment; T5=37 days of rearing and end of the treatment; ST10= 42 days of rearing and 5 days after the end of the treatment; ST20=47 days of rearing and 15 days after the end of the treatments.

rearing and starting time of the treatment; T5=37 days of rearing and end of the treatment; ST10= 42 days of rearing and 5 days after the end of the treatment; ST20=47 days of rearing and 15 days after the end of the treatments. Among the most abundant genes, which were multidrug resistance genes, aminoglycoside and tetracycline resistance genes, the tetracycline resistance genes (tetA and tetW) significantly increased in a dose dependent manner in both low dose and therapeutic dose groups 15 days after the end of the treatment, while several multidrug resistance genes significantly decreased in the therapeutic dose group during the treatment.

At taxonomic level, the metagenomic data showed that Proteobacteria was the predominant phylum in the whole population **(Figure 6a)** but the therapeutic dose of antibiotic decreased its abundance from 83% in the control and low dose group up to 75% in the therapeutic dose group (Fig. 6b), mainly because there was a drop of the Escherichia/Shigella genera from 70% before the treatment to 58% after the treatment (Fig. 6c). Since the metagenomic data indicated that Escherichia was the major host for the multidrug resistance genes at all sampling times, the inhibition of Escherichia in the group treated with the therapeutic dose of chlortetracycline was the primary reason for the decrease of multidrug resistance genes in that group, while the emergence of tetracycline resistance genes was due to the emergence of a new set of bacteria hosting those genes, including Bifidobacterium during and after the end of the treatment.

Overall, these data indicate that the changes in the structure of antibiotic-induced faeces microbial communities accompany changes in the abundance of bacterial hosts carrying specific ARGs in the faeces microbiota and findings like these should contribute to optimize therapeutic schemes for the effective treatment of antibiotic resistant pathogens in poultry farms.

Metagenomic investigation of poultry carcasses

Metagenomic investigations of food microbiota have been less reported in the literature, perhaps because microbial communities of food are generally considered to have a low richness in terms of diversity (Kergourlay et al., 2015). However, the development of NGS technologies and their application in the field of food ecosystems revealed that these communities are perhaps richer than expected and that some of them might play a yet unknown role (Kergourlay et al., 2015).

De Cesare et al. (2017b) applied metagenomics sequencing to preliminary investigate the microbiological profile of chicken carcasses collected from animals fed with different diets. A total of 15 carcasses were collected at the slaughterhouse at the end of the refrigeration tunnel from chickens reared for 35 days and fed with a control diet, a diet supplemented with 1500 FTU/kg of commercial phytase and a diet supplemented with 1500 FTU/kg of commercial phytase and 3g/kg of inositol. Sequence analysis showed that Proteobacteria and Firmicutes represented more than 98% of whole bacterial populations associated to carcass skin in all groups but their abundances were different between tested groups. Moreover, Moraxellaceae and other degradative bacteria showed a significantly higher abundance in the control compared to the treated groups. At species level, Clostridium perfringens showed a relative frequency of abundance significantly higher in the group fed with phytase and Salmonella enterica in the group fed with phytase plus inositol. Although it was not possible to make a correlation between what the chicken ate and the composition of their microflora, this study is one of the first example of metagenomic sequencing of chicken breast and neck skin sampled according to what it is requested in the EU Regulation 2073 on microbiological criteria on foodstuffs and showed statistically significant differences between the metagenomes associated to carcasses obtained from chickens fed with different diets.

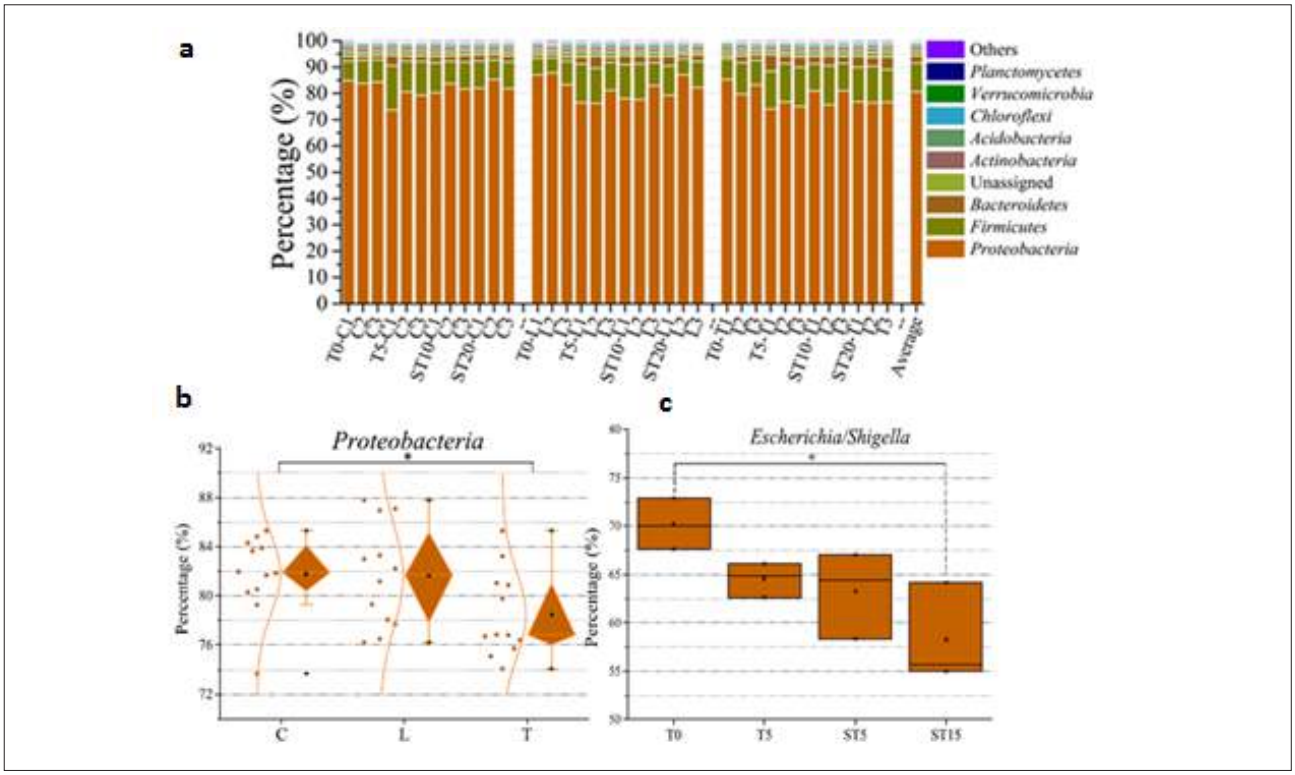

Fig. 6. Phyla identified in the samples tested among which Proteobacteria is the predominant one (a). Decrease of Proteobacteria among the different groups of samples: (control (C); low dose (0.2 g/L) (L); therapeutic dose (2 g/L) (T)) (b). Decrease of Escherichia/Shigella over time in the therapeutic dose group.

In terms of food regulations, Yang et al. (2016) recognize that metagenomic approach has a great utility for investigating the ecology of foodborne pathogens in the food ecosystems but there are still barriers to use shotgun metagenomics for their identification and quantification for regulatory purposes because of a possible misclassification of the microorganisms inherent to the read length; because there are problems to get deep coverage of the pathogenic organisms in the sample due to the presence of other prokaryote and eukaryote within the sample; and also because a comprehensive database containing all possible pathogens does not exist. However, at international level there are research projects like COMPARE (www.compare-europe.eu) which are investigating how to effectively solve all these technical gaps. Besides, there are research projects like CIRCLES (https://circlesproject.eu/) which aims to support the changes needed for sustainable, resilient, competitive, diverse, responsible and inclusive food systems, including chicken meat, by assessing the potential of microbes associated to poultry at whatever level using shotgun metagenomics.

Conclusion

In the literature there are more and more studies addressing the role of the gut microbiome in chicken productivity and health as well as poultry meat safety. Even though our knowledge of the gut microbiome composition, its metabolic functions and influence on animal health is far from complete, metagenomic sequencing seems to be one of the most effective research strategies to fill this gap of knowledge.

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Growth-related breast meat abnormalities in Broilers

Abstract
Artificial selection for fast-growing and high-breast-yield hybrids has considerably marked up the pressure on breast muscle development, leading to the appearance and expansion of myopathies (i.e. White Striping, Wooden Breast and Spaghetti Meat) affecting the pectoral muscle of heavy and fast-growing birds. Growth-related breast meat abnormalities negatively impact both visual aspect and technological properties of raw and processed meat, causing relevant economic damages for the poultry industry. The article aims to provide an overview on the current knowledge about their impact on meat quality, the possible causative mechanisms and forthcoming methods for mitigation.

Keywords
broiler, fast-growing, breast abnormalities, white striping, wooden breast, spaghetti meat, meat quality.

Evolution of the chicken meat market
Regardless different religions, cultures and traditions, poultry meat is one of the most widely eaten animal source food all over the world. In recent decades, the global consumption of chicken meat has rapidly and noticeably increased and recent FAO forecasts show that it's expected to continue to rise (FAO, 2018). This trend has mainly been driven by human population growth and affordability of chicken meat, which is low in fat, rich in high-quality proteins and faces up to religious and cultural issues (Petracci et al., 2014). As a consequence, in order to meet the growing consumer demand, meat market has undergone an intense upsurge in poultry meat production, which since 1960 has increased fivefold and it's expected to further grow by 3.6% per annum from today to 2030 (Steinfeld et al., 2006). Furthermore, global consumer aptitudes over years have been shifted from the consumption

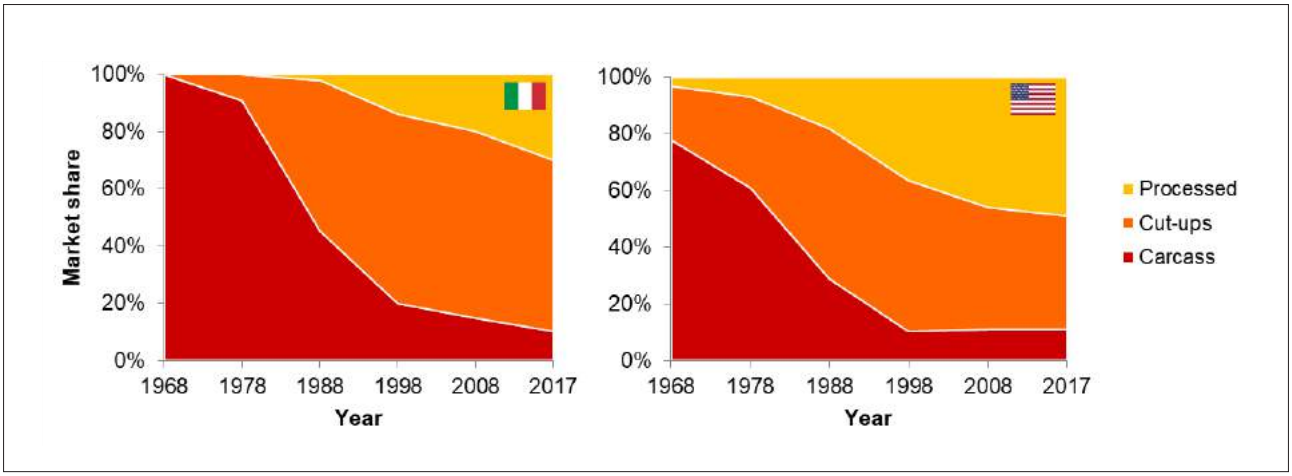

Figure 1. Evolution of market segments and forms of chicken meat in Italy (Unaitalia, 2019) and U.S. (NCC, 2019).

of whole carcass to ready-to-eat and processed products. While in the 1960s the commercialization of the whole carcass represented about 80% of the U.S. market, it was less than 10% in 2016, because nowadays consumers are willing to pay for the convenience of smaller portions already deprived of bone and skin. A similar trend has been observed in the EU, even though the Italian market is still focused on the commercialization of cut-ups **(Figure 1).** In this regard, poultry industry has been inevitably forced to apply intensive selection procedures aimed at accelerating the growth rate and enhancing the muscle mass of animals (Petracci and Berri, 2017).

Currently, chickens designed for meat production usually reach market weight (2.8 kg) within 47 days, namely about half the time compared to 40 years ago, while chickens average daily weight gain is doubled in the past 50 years **(Figure 2)** (NCC, 2019). The substantial genetic progress of the past few decades has resulted in an increased size and meat yield of the breast muscle, which currently exceeds one-fifth of the weight of the bird and certainly represents the most valuable portion in broiler industry **(Table 1)** (Petracci et al., 2015). Within this context, the improvement in broiler meat production has coincided with the development and expansion of

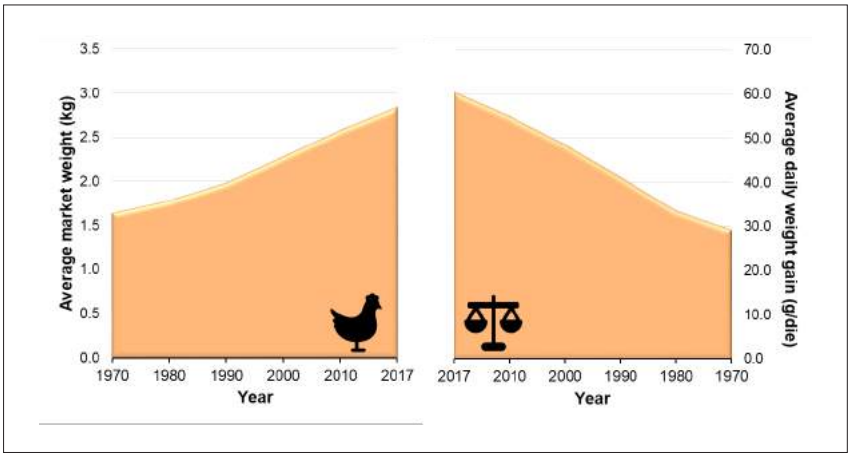

Figure 2: Development of average broiler market weight (kg) and daily weight gain (g/die) in U.S. from 1970 to 2017 (NCC, 2019).

muscular defects affecting the Pectoralis major muscle of fast-growing broiler chickens. Among these, White Striping, Wooden Breast and Spaghetti Meat are the main myopathies that, alone or combined, currently affect breast muscles and negatively influence both visual aspect and technological properties of raw and processed meat, causing relevant economic troubles for the poultry industry.

Muscle growth-related abnormalities: an overview

In recent years, a new group of muscular abnormalities has emerged, being a growing concern for the scientific community. **Figure 3** shows the distinctive traits of White Striping (WS), Wooden Breast (WB) and Spaghetti Meat (SM) myopathies.

WS is macroscopically characterized by the presence of white striations of variable thickness and parallel to muscle fiber direction. White stripes usually cover the cranial part of the breast muscle and, depending on the severity grade, might extend until the caudal region of the fillet. The striations appear like "scars" and have been mainly identified as an accumulation of lipids (lipidosis) and connective tissue (fibrosis) (Kuttappan et al., 2013a). Micro-

[1] AV-Definition according to Commission Directive 2001/82/EC: inactivated immunological veterinary medicinal products which are manufactured from pathogens and antigens obtained from an animal or animals from a holding and used for the treatment of that animal or the animals of that holding in the same locality.

Table 1: Progress in breast yield in a main commercial broiler hybrid (Ross 308 males) from 2001 to 2017.

Year	Body weight (kg)	Age (d)	Breast yield (%)
2001[1]	2.207	43	15.8
2007[2]	2.200	36	18.6
2012[2]	2.200	35	21.1
2014[2]	2.200	34	21.5
2017[2]	2.200	34	22.0

1 (Havenstein et al., 2003)
2 Ross 308 Broiler Performance Objectives.

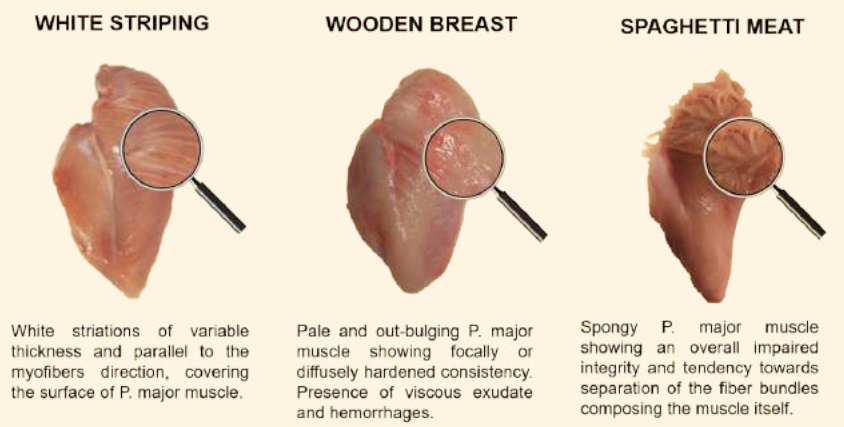

Figure 3: Macroscopic appearance and main common traits of broiler Pectoralis major affected by growth- related meat abnormalities.and U.S. (NCC, 2019).

scopically, histopathological changes associated with WS mainly are necrosis and lysis of fibers, variable cross-sectional area (sign of the existence of degenerating and regenerating fibers) and lymphocytes and macrophages infiltrations (Sihvo et al., 2014; Baldi et al., 2018).

WB myopathy occurs as a focally or diffusely hardened consistency of P. major muscle, which appears pale, rigid, swollen and may have viscous exudate and hemorrhages on its surface (Sihvo et al., 2014). WB and WS often take place concurrently within the same muscle and share common histological features (Baldi et al., 2018). Generally, the extreme collagen deposition along with the accumulation of cross-linked collagen fibrils might be cited as the causes of the stiffness of WB muscles (Soglia et al., 2017; Velleman et al., 2017).

On the contrary, SM condition gets its name from the threadlike and detached muscle fiber bundles composing P. major, which appears mushy, sparsely tight and similar to spaghetti pasta. Microscopically, SM fillets show inflammatory cells infiltrations and a rarefaction of the endo- and peri-mysial connective tissue which appears loose (immature), thus leading to the muscle fiber detachment from each other (Baldi et al., 2018).

Although these muscular defects are easily discernible by several macroscopic distinctive traits, microscopic observations have shown shared histological features (Soglia et al., 2018). Thus, it might be hypothesized a mutual underlying mechanism responsible for their occurrence, even though the precise etiology is yet to be elucidated. It has been recently demonstrated that the occurrence of the aforementioned myopathies is not related to the existence of a specific gene (Pampouille et al., 2018), rather hypoxia seems to play a key role in promoting these muscular defects (Mutryn et al., 2015; Abasht et al., 2016). Undoubtedly, the intensive selection practices carried out during the past decades extremely altered broiler muscle metabolism (i.e. shift toward glycolytic pathway) and architecture (i.e. increased muscle fiber diameter and number, reduced capillary density, etc.) (Petracci et al., 2019). Since hypertrophic muscles are characterized by a faulty muscular oxygenation system and displacement of waste metabolic products, reactive oxygen species accumulate in the muscle and lead to inflammatory processes (Petracci and Berri, 2017). From there on, muscle cells try to contrast inflammation. Sooner or later, myodegeneration overtakes the regenerative capacity of the muscle, thus resulting in the development of muscular issues (i.e. accumulation of fat and/or collagen tissue), peculiar traits of the aforementioned myopathies (Petracci et al., 2019).

Impact of myopathies on meat quality and related costs

Meat affected by growth-related myopathies is usually considered harmless for human nutrition, since no specific biological or chemical hazards have been found to be related to its consumption. However, WS, WB and SM myopathies were found to negatively affect both quality traits and technological properties of raw and processed meat.

[2] Regulation of the European Parliament and of the Council on veterinary medicinal products and repealing Directive 2001/82/EC (PE-CONS 45/18, 14 November 2018).

Table 2 shows a summary of the main implications of myopathies on meat proximate composition and technological properties. Depending on the severity of the defect and the eventual co-existence within the same muscle, muscular abnormalities cause important modifications on meat chemical composition, since their occurrence is generally associated to an overall higher amount of moisture, fat and collagen to the detriment of proteins (Kuttappan et al., 2012a; Mudalal et al., 2015; Soglia et al., 2016b). However, the occurrence of WB abnormality seems to exert a more remarkable effect on meat quality than the mere presence of WS or SM (Baldi et al., 2019). The alterations in meat chemical composition are mainly due to the degenerative processes taking place in the muscle during the injury, as the replacement of fibers by both adipose tissue and collagen and the increase of extra-cellular water because of the inflammatory processes (i.e., edemas) (Clark and Velleman, 2017; Baldi et al., 2019). As a result of the increased fat content and the elevated collagen-to-total-protein ratio, abnormal meats are characterized by a significantly lower nutritional value (Petracci and Berri, 2017). In spite of the higher pHu, meat affected by myopathies also exhibit reduced technological properties (Bowker and Zhuang, 2016). In detail, several authors reported their poor ability to hold water (Mudalal et al., 2015; Tijare et al., 2016; Tasoniero et al., 2017). Among abnormal meats, WB fillets display a particularly remarkable impaired ability to hold both added (i.e. lower marinade uptake and higher cooking loss) and constitutional water (i.e. higher drip loss) (Bowker et al., 2018; Dalgaard et al., 2018), as a result of the severe degeneration of muscle tissue. It has been also speculated that the reduced water holding and binding capacities of abnormal meat could be due to an overall reduction in protein functionality, since myopathic muscles show a higher concentration of oxidized protein (Utrera and Estévez, 2012; Soglia et al., 2016a). The textural properties of raw meat were found to be altered by muscular abnormalities, with a special reference to WB meat that, if compared to unaffected samples, exhibits significantly higher shear and compression forces (Chatterjee et al., 2016; Soglia et al., 2017), while WS and SM conditions sparsely affect texture of both raw and cooked meat (Kuttappan et al., 2013b; Baldi et al., 2019). According to the analytical method, controversial results were found for cooked WB meat. While (Aguirre et al., 2018) detected significantly higher hardness of WB samples using texture profile analysis, (Soglia et al., 2017) and (Baldi et al., 2019) didn't find any difference in the compression forces between cooked fillets. The authors assumed that the solubilization of the thermally-labile collagen cross-links is the cause for the tenderization of WB meat during cooking.

Table 2. Main effects of growth-related meat abnormalities on proximate composition and technological properties of broiler breast meat (= very slight effect; ↑ high; ↑↑ very high; ↓ low; ↓↓ very low; n.a= not available).

	WHITE STRIPING	WOODEN BREAST	SPAGHETTI MEAT
Proximate composition			
Moisture	=	↑↑	↑
Protein	↓	↓↓	↓↓
Lipid	↑↑	↑↑	↑
Ash	=	=	=
Collage	=	↑↑	↓
Technological properties			
pHu	=	↑	=
Drip loss	↓	↑	n.a.
Cook loss	↑	↑	n.a.
Ability to absorb marinade solution	↓	↓↓	n.a.
Shear force	=	↑↑	=

Beyond the detrimental effect of muscular abnormalities on meat quality traits and technological properties, WS, WB and SM raise concerns over the consumer acceptance of meat, since their occurrence remarkably impairs the visual appearance of fillets and reduces the consumer´s willingness to buy (Kuttappan et al., 2012b; Huang and Ahn, 2018). Severely affected fillets are usually discarded or downgraded for the manufacture of further processed products (i.e., nuggets, sausages, hamburger), while moderate cases are marketed for fresh retailing (Petracci et al., 2014). Considering the high and unsustainable incidence of these worldwide-spread myopathies, it has been estimated that the defects result in $ 200 million loss per year in the US (Bunge, 2019). The economic damage is related not only to poultry processors (i.e. meat downgrading or discarding, lower processing yields, etc.) but also to retailers. Indeed, as reported by a recent article published in the Wall Street Journal, Wendy's, a colossal fast-food chain in the US, after several customers complaints, decided to shift its chicken

supply to smaller birds, despite the $30 million higher cost for the company (Bunge, 2019). Moreover, it was also mentioned that a US major chicken producer (Sanderson Farms) has begun slaughtering animals at younger ages in order to reduce the frequency of wooden breast myopathy, while Panera Bread and Whole Foods Market declared they would shift their chicken purchasing toward slower-growing genotypes (Bunge, 2019).

Methods for mitigation

Given the notable impact of WS, WB and SM on the quality of both raw and processed meat and the related economic damage, poultry industry and the scientific community take an interest in searching for solutions to avoid or at least mitigate the occurrence of muscular abnormalities. It is commonly recognized that the incidence of myopathies boosts with increasing growth rate, slaughter age and weight (Lorenzi et al., 2014; Radaelli et al., 2017; Kuttappan et al., 2017). Thus, attempts have been made in the field of animal nutrition to reduce the occurrence of abnormalities through the modulation of both feed formulation (i.e. dietary supplementation of antioxidants, organic minerals, vitamins and aminoacids) or dietary intake through feed restriction. However, the implementation of these strategies under commercial conditions might be challenging and does not result in any significant mitigation effect, because a reduction of the incidence of breast abnormalities has been attributed as an indirect consequence of decreased slaughter weight and breast size of the animals (Petracci et al., 2019). Despite it was recently assessed that an increased arginine:lysine ratio can have significant mitigation effect on breast meat abnormalities (Zampiga et al., 2018), further researches are needed to confirm these outcomes. Within this context, the most efficient solution seems the incorporation of downgraded meat into the formulation of processed products. Finely or coarsely minced WB meat could be included in the formulation of hamburger and meatballs without detrimental effects on finished product quality (Brambila et al., 2017; Xing et al., 2017). Since it has been proved that muscular abnormalities mainly affect the superficial section of breast muscles (Baldi et al., 2018, 2019), one possible approach could be to separately process superficial and deep layers of fillets to limit the effect of meat downgrading (Petracci et al., 2019). However, no really efficient solutions aimed at inhibiting the onset of myopathies or at least alleviating the symptoms and consequences on raw and processed meat quality have been found yet. Furthermore, it seems that genetic selection for broilers growth has reached a plateau and further improvements might be restrained by muscle biological potential and animal welfare concerns (Tallentire et al., 2018). In this scenario, it has been recently suggested that particular attention should be given on the modulation of embryonic formation of additional myofibers, instead of relying on post-hatch selection aimed at increasing muscle mass accretion (Velleman, 2019).

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NOTES

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Foodborne diseases and poultry production

Abstract

In spite of significant improvement in technology and hygienic practices in developed countries at all stages of poultry production foodborne diseases remain a persistent threat to human and animal health. Beside the current legislations, the main strategy to control microbial food borne hazards should include Good Animal Husbandry Practices (GAHPs) at the farm level through sound hygienic measures, applied in poultry houses, environment and feed manufacture, as well as reducing colonization with feed additives, competitive exclusion, treatment or vaccines. Furthermore, hygienic measure should also be considered during transport and slaughtering. In all cases, surveillance and monitoring programmes must be adapted and followed strictly in aim to allow early intervention. In addition, the development of antibiotic resistant bacteria will also be a continuous public health hazard.The present paper describes the main strategy to control food borne infections in poultry, with special attention to European legislations toward safe poultry meat.

Keywords

Poultry, foodborne diseases, salmonella, campylobacter, antibiotic resistance, regulations

Introduction

In spite of significant improvements in technology and hygienic practices at all stages of poultry production in developed countries, accompanied by advanced improvement in public sanitation, foodborne diseases remain a persistent threat to human and animal health. Food borne diseases are still big issues of major concern in those countries. In developing countries, the need to produce sufficient food to meet the requirements of population increases, accompanied by bad economic situations often overshadow the need to ensure safe food products. Regardless of this fact, safe food is a fundamental requirement for all consumers, rich or poor. Food safety is not a discovery of recent

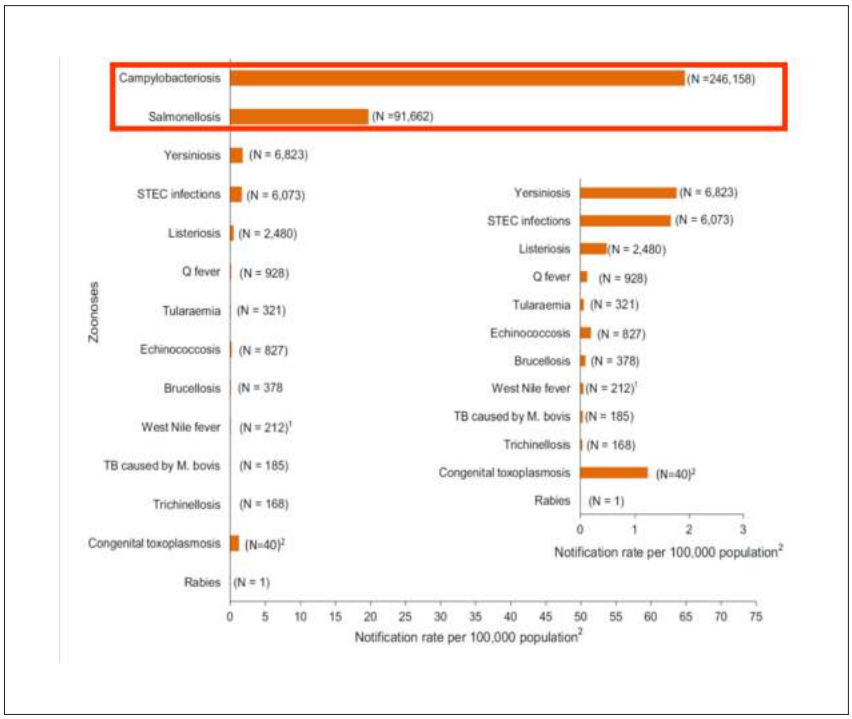

Figure 1: Food-borne Diseases in humans in 2017 in the EU(EFSA, December 2018)

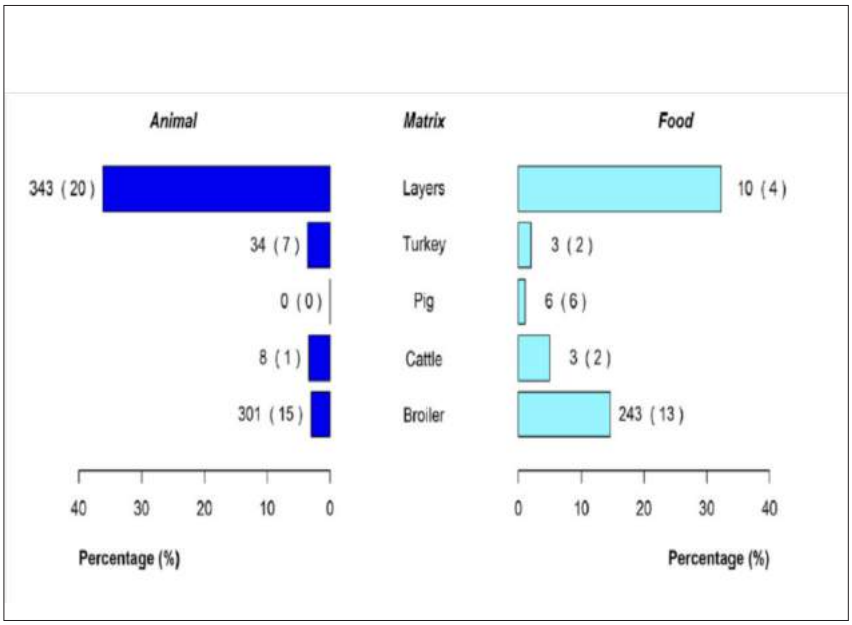

Figure 2: Distribution of S. Enteritidis among food and animal sources in the EU, 2017 (EFSA, December 2018)

times; it is a natural basic instinct of human survival. During human evolution, several approaches were adopted to achieve safety of food. One of the most famous approaches was practiced by several kings, which would employed official and well trusted „tasters" that served as food safety sentinels for the kings and royal family members. Food safety and quality of food are currently big issues of major concern.

Many reports during recent years have shown that Salmonella and Campylobacter spp. are the most common causes of human foodborne bacterial diseases linked to poultry **(Figure 1)**. In some areas also verotoxin producing Escherichia coli 0157:H7 (VTEC), Listeria and Yersinia have surfaced as additional foodborne pathogens causing human illness. Several other toxicogenic bacterial pathogens, such as Staphylococcus aureus, Clostridium perfringens, Clostridium botulinum and Bacillus cereus can also enter the human food chain via contaminated poultry carcasses. In addition, the development of antibiotic resistance in bacteria, which are common in both animals and humans, such as Methicillin Resistant Staphylococcus aureus (MRSA) and Extended-spectrum beta-lactamase (ESBL) bacteria, are also an emerging public health hazard.

Salmonella infection

Salmonella infections in poultry are distributed worldwide and result in severe economic losses when no effort is made to control them. In poultry, the genus Salmonella of the family Enterobacteriaceae, which include more than 2500 serovars, can roughly be classified into three categories or groups as follow: Salmonella can also be divided into three groups based on their host specificity and invasiveness (Hafez, 2013). Invasive salmonellas have the capability to "invade" the body from the intestinal lumen and thus infect organs, causing more serious disease. Group 1 contains serovars, which are highly host adapted and invasive. Examples are S. Gallinarum and S. Pullorum in poultry or S. Typhi in humans. Group 2 contains non-host adapted and invasive serovars. Salmonella in this group are of most concern regarding public health, since some of them are capable to infect humans and food producing animals and especially poultry can

serve as reservoirs. There are approximately 10 – 20 serovars in this group. Currently, the most relevant serovars of them are S. Typhimurium, S. Enteritidis, S. Heidelberg, S. Hadar as well as S. Arizonae. **Figure 2** shows the distribution of S. Enteritidis among food and animal sources, which are closely related.

Group 3 contains non-host adapted and non-invasive serovars, which are harmless for animals and humans. Most serovars of the genus salmonella belong to this group. Some serovars may be predominant for a number of years in a region or country. Then, they disappear and are replaced by another serovars (Hafez and Hauck, 2016). The infection can be transmitted vertically through contaminated eggs laid by infected carriers as well as horizontally spread (lateral). Hatcheries are one of the major sources of early horizontal transmission. Horizontal spread of Salmonella occurring during the hatching was shown in chickens, when contaminated and Salmonella-free eggs were incubated together. Salmonella can also spread through the hatchery by means of contamination of ventilation ducting, belt slots or door seals within hatchers, but may also result from infection and contamination that continuously recycles between hatchers, hatched birds, dust and crate washing equipment. During rearing the infection is transmitted horizontally (laterally) by direct contact between infected and uninfected flocks, and by indirect contact with contaminated environments through ingestion or inhalation of Salmonella organisms. Subsequently, there are many possibilities for lateral spread of the organisms through live and dead vectors. Transmission frequently occurs via faecal contamination of feed, water, equipment, environment and dust in which Salmonella can survive for long periods. Failure to clean and disinfect properly after an infected flock has left the site can result in infection of the next batch of birds. Significant reservoirs for Salmonella are man, farm animals, pigeons, waterfowl and wild birds. Rodents, pet's insects and litter beetles (Alphitobius diaperinus) are also potential reservoirs and transmit the infection to birds and between houses (Roche et al., 2009). Probably one of the most common sources for lateral spread of the organisms is feed. Nearly every ingredient ever used in the manufacture of poultry feedstuffs has been shown at one time or another to contain Salmonella. The organism occurs most frequently in protein from animal products such as meat and bone meal, blood meal, poultry offal, feather meal and fishmeal. Protein of vegetable origin has also been shown to be contaminated with Salmonella (Hafez et al., 1997; Dutta et al., 2010).

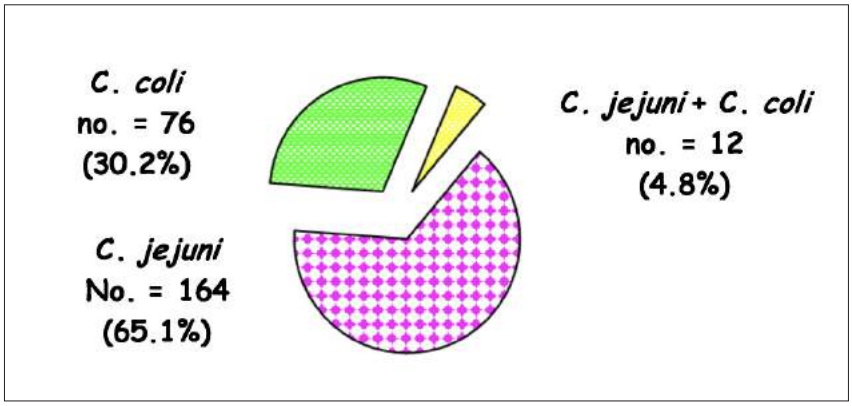

Figure 3: Campylobacter in a meat turkey flock (Düpre et al., 2010)

Since November 2003, several regulations from the European Parliament Council Regulation on the control of salmonella and other specified food-borne zoonotic agents were passed. This regulation covers the adoption of targets for the reduction of the prevalence of specified zoonosis in animal populations at the level of primary production, including breeding flocks (Chickens and turkeys), layers, broiler and turkey flocks. Food business operators must have samples taken and tested for the zoonosis and zoonotic agents especially Salmonella **(Table 1)** as summarized by Hafez (2010).

Campylobacters

Thermophilic campylobacters are the most common bacterial cause of diarrhoea in humans worldwide. Enteric diseases caused by the thermophilic species C. jejuni, C. coli, C. lari, and C. upsaliensis range from asymptomatic infections to severe inflammatory bloody diarrhoea. The natural habitat of thermophilic Campylobacter is the intestinal tract of healthy birds and raw meat that can be contaminated during the slaughtering process (EFSA, 2015). It is estimated that as many as 90% of broilers and turkeys may harbour Campylobacter while showing little or no clinical signs of illness (Sahin et al., 2002). Hafez et al. (2001) investigate 10 turkey flocks for the presence of Campylobacter. Faecal samples were collected weekly from 1st week of age until slaughter. All monitored turkey flocks were positive for Campylobacter. Three flocks (30.0%) appeared to be infected with only one biotype. In the other 7 flocks (70.0%) two or more different biotypes were isolated during the rearing period and in slaughtered house too. In addition, Düpre et al. (2010) examined bacteriologically 1167 boot swabs taken from 161 fattening turkeys for Campylobacter spp. Campylobacter spp. were iso-

Table 1: Minimum sampling requirements for different Salmonella species in breeding flocks, layers, broilers and turkeys (Hafez, 2010)

Zoonoses or zoonotic agent	Animal population	Time of Sampling by food business operators
Breeding flocks of Gallus gallus (EC, 2005)		
S. Enteritidis, S. Typhimurium S. Hadar S. Infantis S. Virchow	rearing flocks	→ day-old chicks → four-week-old birds → two weeks before moving to laying phase or laying unit at the holding or at the hatchery
	adult flocks	→ every second week during the laying period
Laying hens (EC, 2006a)		
S. Enteritidis, S. Typhimurium	rearing flocks	→ day-old chicks → pullets two weeks before moving to laying phase or laying unit
	laying flocks	→ every 15 weeks during the laying phase
Broilers (EC, 2007b)		
S. Enteritidis, S. Typhimurium	broilers	→ within three weeks before the birds are moved to the slaughterhouseare moved to the slaughterhouse
Turkey breeders (EC, 2008)		
S. Enteritidis, S. Typhimurium	rearing flocks	→ day-old chicks → four-week-old birds → two weeks before moving to laying phase or laying unit
	adult flocks:	→ at least every third week during the laying period at the holding or at the hatchery
Fattening turkeys (EC, 2008		
S. Enteritidis, S. Typhimurium	turkeys	→ within three weeks before the birds are moved to the slaughterhouse

lated from 56 % of the investigated farms. 65.1 % of isolates were C. jejuni and 30.2% were C. coli. From 4.8% of positive samples C. jejuni as well as C. coli were isolated (Fig 3). Furthermore, Ahmed et al. (2016) examined the prevalence, genotyping and risk factors of thermophilic Campylobacter spreading in organic turkey farms in Germany. Campylobacter spp. were detected in cloacal swabs in all 5 turkey flocks with prevalence ranging from 90.0 to 100 %. 13 cloacal swabs from birds in two farms harboured mixed population of thermophilic campylobacters. In total, from 158 Campylobacter isolated from turkeys 89

Table 2: Prevalence and genotyping of Campylobacter spp. isolated from organic turkey farms (Ahmed et al., 2016)

	Farm 1	Farm 2	Farm 3	Farm 3	Farm 5
No. of birds	1003	1100	2000	1400	1500
Age in weeks	8	8	8	4	6
Turkey line	Kelly	Kelly	B.U.T. 6	B.U.T. 6	B.U.T. 6
Cloacal swabs	30 /30	38/38	27/30	35 /30	30/30
Water samples	1	negative	2	negative	negative
positive black beetles	negative	negative	3	negative	negative
No. C. jejuni	8	17	19	5	20
Prevalence of C. jejuni (%)	26.67	56.67	63.33	14.29	66.67
No. C. coli	22	19	8	30	10
Prevalence of C. Col (%)	73.33	63.33	26.67	85.71	3333
C. jejuni Genotype	3	2	4	1	5
C. coli Genotype	2	4	1	2	4
Total	100 %	100 %	90 %	100 %	100 %

(56.33 %) were identified as C. coli and 69 (43.76 %) as C. jejuni. Three Campylobacter (2 C. jejuni and 1 C. coli) were detected in drinkers of two farms and 3 C. coli were isolated from darkling beetles of one farm. No Campylobacter were isolated from main water tanks. The genotypes of campylobacters isolated from water samples or beetles were identical with those isolated from turkeys. **(Table 2)**.

Poultry and poultry products remain the most common source of foodborne human campylobacteriosis. The major route for Campylobacter infection in poultry appears to be the horizontal transmission from the environment. Specific flocks that become infected show rapid rate of intra-house transmission and a high isolation rate from caecal swabs, water and litter. Campylobacter spp. are widespread in poultry not only during the growing period, but also on the poultry meat during slaughter and during processing of poultry products. Horizontal transmission is the most important mode of the introduction of Campylobacter into poultry flocks. However, the ability of Campylobacter to spread is limited by their relatively low tenacity, which can vary between strains. Especially dry environments kill Campylobacter within one or two hours (Evans and Sayers, 2000).

Antibiotic resistant

The development of antibiotic resistance in bacteria, which are common in both animals and humans, is an emerging public health hazard. Controlling of the above mentioned foodborne micro-organisms requires a broader understanding of how microbial pathogens enter and move through the food chain, as well as the conditions that promote or inhibit growth for each type of organism.

Multi-resistant bacteria are increasingly posing a hazard to human and animal health worldwide, impeding successful antibacterial treatment (Arias et al., 2010; EFSA, 2017). In addition, the development of novel antibiotics does not keep step with the emergence of antimicrobial resistance in bacteria (García-Rey, 2010).

El- Adway et al. (2012) investigated 76 Camyplobacter jejuni isolates recovered from 67 epidemiologically unrelated meat turkey flocks in different regions of Germany in 2010 and 2011. Only one isolate was sensitive to all tested antibiotics. The numbers of isolates that were sensitive to streptomycin, erythromycin, neomycin, and amoxicillin were 69 (90.8%), 61 (80.2%), 58 (76.4%), and 44 (57.9%), respectively. The emergence of a high resistance rate and multidrug resistance to three or more classes of antimicrobial agents were observed. The resistance against sulphamethoxazole/trimethoprim, metronidazole, ciprofloxacin, nalidixic acid, and tetracycline was 58 (76.3%), 58 (76.3%), 53 (69.7%), 51 (67.1%), and 42 (55.3%), respectively. Multidrug resistance to three or more classes of antimicrobial agents was found and ranged from 3.9% to 40.8%. Similar results were also found by examination of isolates collected from

different free-range turkey flocks in Germany (El-Adway et al., 2015).

ESBL-producing Enterobacteriaceae have emerged as pathogens in both poultry and humans (Bradford, 2001, Rawat and Nair, 2010, Moawad et al., 2018).

Among multi-resistant bacteria, vancomycin-resistant enterococci (VRE) have been estimated as one of the most common bacteria causing a rise in cases of nosocomial infections in humans in the last few years (Arias et al., 2010). The prevalence of VRE in 20 turkey flocks reared in the southwest of Germany was investigated. Enterococci were tested on the presence of the vancomycin resistance genes vanA, vanB (B1/B2/B3), and vanC (C1/C2/C3). Vancomycin-resistant enterococci were detected in 15 (75%) of the 20 turkey flocks investigated. In a total of 68 isolates from birds and dust samples, enterococci bearing van-genes were detected. Of these, 12 isolates carried the vanA gene (17.6%) and 56 isolates carried the vanC1 gene (82.6%). Neither vanB (B1, B2, B3) genes nor the vanC2 or vanC3 genes could be detected (Sting et al., 2013).

Maasjost et al. (2015) investigated the antimicrobial susceptibility patterns of Enterococcus faecalis and Enterococcus faecium isolated from poultry flocks in Germany and they found that high resistance rates were identified in both Enterococcus species for lincomycin (72%–99%) and tetracycline (67%–82%). Half or more than half of Enterococcus isolates were resistant to gentamicin (54%–72%) and the macrolide antibiotics erythromycin (44%–61%) and tylosin-tartate (44%–56%). Enterococcus faecalis isolated from fattening turkeys showed the highest prevalence of antimicrobial resistance compared to other poultry production systems.

In addition, Livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) have been isolated from a number of livestock species and persons involved in animal production. Turkey meat was also showed to be contaminated with MRSA (De Boer et al., 2009). Richter et al. (2012) investigated the prevalence of LA-MRSA in fattening turkeys and people living on farms that house fattening turkeys. MRSA was detected in 18 (90%) of the 20 turkey flocks investigated. Out of examined 59 nasal swabs from persons working on the farms 22 samples were MRS-positive (37.3%). None of these persons showed clinical symptoms indicative of an MRSA infection. People with frequent access to the stables were more likely to be positive for MRSA. Similar results were about MRSA in turkeys were published by El-Adway et al. (2016).

General approaches to control food borne infections

To control the food borne organisms, information is required to understand more fully, how microbial pathogens enter and move through the food chain, and the conditions, which promote or inhibit growth for each type of organism. In general, the main strategy to control food borne infections in poultry should include monitoring, cleaning the production chain from the top, especially for vertically transmitted microorganism such as Salmonella by culling infected breeder flocks, hatching egg sanitation and limiting introduction and spread of infections at the farm level through effective hygiene measures (Hafez, 1999, 2005, Mueller-Doblies et al., 2010). An intensive and sustained rodent control is essential and needs to be well planned and routinely performed and its effectiveness should be monitored. In addition, reducing bacterial colonization by using feed additives such as short chain organic acids (formic acid, propionic acid), carbohydrates (lactose, mannose, galactose, saccharose), probiotics, competitive exclusion (Schneitz, 2005, Vicente et al., 2007) or use of vaccines are further possibilities. Live and inactivated vaccines are used to control Salmonella in poultry (Gast, 2013). Generally, vaccination alone is of little value, unless it is accompanied by improvements in all aspects of management and biosecurity. In addition, further attention must be paid to the development of efficient vaccines against campylobacter infections.

Since the success of any disease control programme depends on the farm and personal sanitation, it is essential to incorporate education programmes about microorganisms, modes of transmission as well as awareness of the reasons behind such control programmes by people involved in poultry production. In addition, effective education programmes must be implemented to increase public awareness of the necessary measures to be taken for protection against bacteria in food products from poultry.

In spite of significant improvements in technology and hygienic practices at all stages of food production, accompanied by improvement in public sanitation, food borne infections remain a persistent threat to human and animal health. Since many humans fail to apply hygienically acceptable food handling and cooking practices, and since processing plants are not able to reduce the level of pathogenic bacteria in poultry products, every effort must be made to reduce the Salmonella contamination of live birds before despatch to processing plants. New approaches to the problem of contamination must be adopted and the discussion on the decontamination of the end product must be re-evaluated carefully and without emotion. In addition, research must continue to find

additional control and preventive means. As a long term contribution, poultry lines genetically resistant to some pathogens should be developed.

Conclusions

Toward food safety in the EU several legislations are into force and their aims can be summarized according to Mulder (2011) as follows:

1. Safety (consumer health): by new methods to reduce the use of antibiotics /medicines; improve disease resistance; zoonosis control; traceability of animals and products

2. Safety (product safety): stimulate and control hygienic processing, traceability of products and materials intended to come into contact with food

3. Animal welfare: animals kept according to rules/systems

4. Product quality: improved quality and composition; quality and chain control systems; traceability of animals and products.

5. Environment: reducing environmental contamination, Nitrogen and Phosphorous. There is a critical look at the use of by-products of human food production. The re-use of by-products for non-food applications (feathers) should be encouraged.

6. Rural impact, economic effects and bio-diversity

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Performance of current Peking duck breeds

Abstract

Data on the performance of commercial ducks are rare and outdated in most cases. It was the objective of the present test to explore the performance criteria and carcass characteristic of commonly used duck breed. The results may be used as up-to-date reference values. A total of 2380 ducks of 5 different breeds were raised from day-old to 42 days of age. The birds were fed a 3-phase feeding program. Body weight and feed consumption were recorded in weekly intervals. Mortality was recorded daily. Samples of all breeds were slaughtered at 36, 40 and 42 days of age. Slaughter weight and percentage of different parts of the carcass were determined. Pooled means and minimum/maximum values are shown of the present experiment. Data of earlier performance tests (2004 and 2008) are reported to show the temporal changes. At the end of the test (42 days) live weight was 3.6 kg and feed conversion rate 2.03. The ducks reached 3 kg live weight at 36 days of age, 10 and 8 days earlier than in 2004 and 2008. Daily weight gain was 85 g and feed conversion rate 1.8 kg/kg. Percentage of the breast including skin increased from 20.7 % at 36 days to 23.8 % at 42 days. The range of minimum and maximum values was high for all criteria. Mortality from day 7 to 42 was 2.8 %. Compared with data of 2008 there was an annual improvement in slaughter age of – 0.8 days; in daily weight gain of +1.65 g and feed conversion rate of -0.05 kg feed/kg gain. The European Production Index (EPI) and Income Over Feed Cost (IOFC) were calculated for 36, 40 and 42 days of age. Higher slaughter age showed positive economic effects in some lines. The increase in feed conversion rate was obviously balanced by the increased growth rate of the breast muscle. Range of the means, however increases with slaughter age. Optimum age at slaughter will vary in response of the particular characteristics of the breed.

Keywords

Pekin ducks, growth rate, feed conversion, slaughter age, slaughter yield, economics

Indroduction

Planning and calculation of facilities for duck production require reliable basic biological data including growth rate, feed intake, feed conversion rate. These data are also important to appraise the environmental aspects of duck production. The existing data material on duck production is outdated. The last information of the experimental station LVFZ (Lehr-, Versuchs- und Fachzentrum für Geflügel- und Kleintierhaltung, Mainbernheimer Str. 101, 97318 Kitzingen; Germany) on this subject has been published in 2008. There is considerable genetic progress in the performance, feed intake, feed conversion and criteria of the slaughtered birds of commercial duck breeds. It was the objective

Table 1: Nutrients (% of fresh matter) of the starter (day 1 – 14), grower (day 15 – 28) and finisher (day 29 – 42)

	Starter	Grower	Finisher
Crude protein (%)	21,5	14,6	17,5
Crude fat (%)	3,5	3,1	3,5
Crude fibre (%)	2,7	4,8	3,2
Ash (%)	6,1	4,9	4,5
Calcium (%)	1,00	0,85	0,80
Phosphorous (%)	0,65	0,50	0,55
Sodium (%)	0,16	0,18	0,18
Lysine (%)	1,40	0,81	1,10
Methionine (%)	0,60	0,43	0,50
ME (MJ/kg)	12,0	11,5	12,3

Table 2: Means and minimum/maximum across breeds of body weight, cumulated feed and water intake and feed conversion rate from day old to 42 days of age.

Age (days)		Body weight (g)	Feed intake (g)	Water intake (ml)	Feed conversion (kg/kg)
0	x	51.4			
7	x	257	225	566	1.09
	min / max	251 - 275	212 - 252	490.8 - 646.8	1.04 - 1.17
14	x	758	813	2.196	1.15
	min / max	728 - 821	763 - 963	2.067 – 2.479	1.11 - 1.25
21	x	1.393	1.890	4.897	1.41
	min / max	1.353 – 1.497	1.744 – 2.086	4.588 – 5.655	1.31 - 1.44
28	x	2.132	3.345	8.341	1.60
	min / max	2.052 – 2.338	3.093 – 3.763	7.648 – 9.609	1.54 - 1.64
36	x	3.055	5.409	13.628	1.80
	min / max	2.955 – 3.189	4.965 - 6168	12.469 – 16.034	1.77 - 1.96
40	x	3.442	6.637	16.729	1.95
	min / max	3.341 – 3.519	6.093 – 7.457	15.253 – 19.482	1.81 - 2.17
42	x	3.608	7.215	17.991	2.03
	min / max	3.522 – 3.707	6.642 – 8.044	16.512 – 20.846	1.88 - 2.29

of the LVFZ to actualize, in cooperation with MEGA animal nutrition and Duck-Tec Hatchery, the above mentioned criteria of five different commercial duck breeds so as to update the data base for duck producers and governmental authorities.

Birds, material and methods

A total of 2380 ducks of 5 different breeds (Cherry Valley, Maple Leaf, Wichmann, Grimaud Frères and Orvira) were used. Day-old ducklings were housed in floor pens on straw as litter. The experimental house was windowless and force-ventilated. Stocking density was 6 birds/m^2. The birds were fed a 3-phase programme (MEGA Animal Nutrition, 49429 Visbek, Germany) as shown in **table 1**. Starter was fed from day 0 to 14, grower from day 15 to 28 and finisher from day 29 to 42. Body weight and feed consumption were recorded on day 0, 7, 14, 21, 28, 36, 40 and 42. Mortality was recorded daily. Slaughter yield was determined using samples at 36, 40 and 42 days of age. Slaughter weight and the percentage of valuable parts were recorded. The birds were healthy throughout the experiment and no veterinary intervention was necessary.

Results and Discussion Performance

The performance data across the five different breeds are shown in **table 2**. The development of the main performance criteria from 2004 to 2018 are provided in **table 3** for reference. Data from 2008 and 2004 stem from earlier tests under practical conditions and are not directly comparable with the results of the present (2018) station test. The mean daily weight gain and feed conversion rate in 2008 were 68.4 g and 2.32 kg/kg respectively, and in 2004 65.2 g and 2.5 kg/kg resp. The birds reached the slaughter weight of 3.0 kg at 44 days of age in 2008 and at 46 days in 2004. In the present experiment the slaughter weight (3.055 kg) was reached at 36 days of age. Besides the increase of growth rate the feed conversion rate was improved from 2.5 in 2004 to 1.8 in 2018. The annual change during the 10 years from 2008 to 2018 was -0.8 days in slaughter age, +1.65 g in daily weight gain and -0.05 in feed conversion rate. This corresponds to savings of about 150 g feed per duck which represents a considerable improvement of the use of resources and reduction of resource based

Table 3: Development of main performance criteria in 2004, 2008 and 2018 tests and difference between 2008 and 2018

Criteria	2004 *	2008 **	2018	Δt / Jahr
Live weight (kg)	3,0	3,0	3,1	
Age at slaughter (d)	46	44	36	- 0,8
Daily weight gain (g)	65,2	68,4	84,9	+ 1,65
Feed conversion rate (kg/kg)	2,50	2,32	1,80	- 0,052

* Source: S. Graser et al. (2004)
** Source: A. Tischler et al. (2008)

Table 4: Means and min/max values for mortality, European Production Index (EPI), and Income over feed cost (IOFC) from 7 – 36, 40 and 42 days of age

Age (days)		Mortality (%)	EPI (Units)	IOFC (€ / bird)
7-36	x	2,0	449	2,12
	min / max		426 – 466	2,04 - 2,19
7-40	x	2,2	419	2,23
	min / max		377 – 445	2,05 - 2,34
7-42	x	2,8	402	2,27
	min / max		349 – 433	2,00 - 2,40

Early mortality from day 1 to 6 was 2.9%

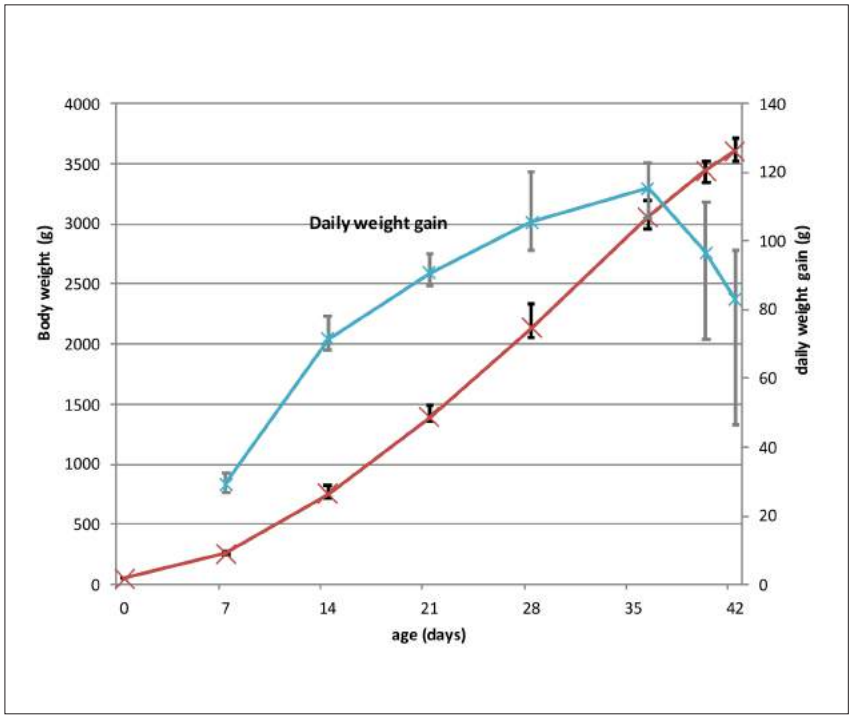

Figure 1 Means (all breeds) and SD of body weight development from day-old to 42 days of age

environmental problems. Figure 1 shows the change of body weight and feed conversion rate over the growth period from day-old to 42 days of age. Body weight follows a normal growth curve for ducks with a sharp increase from day 7 onwards (Figure 1). Daily weight gain increased about 27 g at day 7 to 110 g at 35 days of age. Thereafter daily weight gain is falling towards 80 g at 42 days and the Standard Deviation increases considerably. The increased variation in daily weight gain is probably caused by the sex and breed effect which is gaining momentum with increasing age. It has to be mentioned that in the present study the performance results have been produced under standardized conditions of a research station. The feeding program of the present study was not conceived to achieve highest growth rate. Using

Table 5: Means (across all breeds) and min/max of slaughter weight (g), slaughter yield (%) and for different parts (in g and in % of slaughter weight) of the birds at 36, 40 and 42 days of age

Age (days)		36	40	42
Slaughter weight (g)*	x	2.080	2.409	2.532
	min / max	1992 - 2177	2298 - 2485	2443 - 2590
Slaughter Yield (%) **	x	68,6	69,9	70,6
	min / max	66,8 - 70,1	68,6 - 71,4	69,3 - 72,5
Breast with skin (g)	x	435	559	607
	min / max	335 - 535	447 - 654	526 - 673
Breast skin (%)	x	20,7	23,0	23,8
	min / max	16,6 - 24,5	19,2 - 26,3	20,7 - 26,6
Thigh (g)	x	523	569	585
	min / max	498 - 542	548 - 581	547- 616
Thigh (%)	x	25,2	23,7	23,2
	min / max	498 - 542	548 - 581	547- 616
Wings (g)	x	292	343	360
	min / max	281 - 301		343 - 377
Wings (%)	x	14,1	14,0	14,3
	min / max	13,8 - 14,5	13,7 - 14,9	13,7 - 14,6
Carcass (g)	x	733	847	884
	min / max	705 - 749		854 - 919
Carcass (%)	x	35,3	35,2	35,0
	min / max	34,2 - 37,2	33,7 - 37,4	34,0 - 36,2
Liver (g)	x	84,2	83,5	82,8
	min / max	77,7 - 90,0	76,7 - 87,4	74,5 - 91,2
Liver (%)	x	4,1	3,5	3,3
	min / max	3,9 - 4,4	3,3 - 3,8	3,0 - 3,6
Heart (g)	x	15,6	16,0	17,2
	min / max	14,3 - 18,0	14,1 - 18,2	15,9 - 19,0
Heart (%)	x	0,75	0,66	0,68
	min / max	0,69 - 0,91	0,57 - 0,75	0,62 - 0,78
Gizzard(g)	x	44,6	50,1	50,9
	min / max	37,6 - 53,1	39,3 - 58,6	38,1 - 57,8
Gizzard (%)	x	2,2	2,1	2,0
	min / max	1,7 - 2,6	1,6 - 2,5	1,5 - 2,3

* slaughtered, with neck, without feathers, head, inner organs and paddles
** slaughter weight in paercent of live weight (measured immediately before slaughter)

common commercial duck diets would have given higher growth rates, but on the expense of the bird`s health.

Economics

Table 4 shows means and range of mortality, European Production Index (EPI) and Income Over Feed (IOFC) over the growing periods from 7 to 36, 7 to 40 and 7 to 42 days. The EPI is calculated according to the following formula:

$$EPI = \frac{\text{survivability (\%)} \cdot \text{daily weight gain (g)}}{\text{feed conversion rate (kg of feed per kg of weight gain)}} * 10$$

The assumed feed price for the calculation of IOFC was 27 €/dt and price for the ducks was 1.17€ per kg live weight. The EPI was nearly 450 from day 7 to 36. This corresponds to the average EPI in broilers. Despite higher growth rate of the ducks from day 36 onwards there is a sharp reduction in mean EPI: 419 and 402 when slaughter age increased to 40 an 42 days **(table 4; figure 2).** Since mortality was generally low, this is mainly due to the deterioration of feed conversion with increasing age. The high range however shows that there exists potential for further improvement. Some groups showed an EPI which was substantially higher than 450 from day 7 to 36, and of 450 from 7 to 40 days of age. In contrast to EPI the mean IOFC increased from 36 to 42 days of slaughter age. The difference between the minimum and maximum values increases with from 0.15 to 0.29 and 0.40 € as slaughter age increased from 36 to 40 and 42 days. The development of the minimum and maximum values shows that the increase in IOFC with increasing slaughter age is mainly supported by the groups of maximum economic result. The minimum values show a small negative trend in response to slaughter age.

Slaughter yield

Criteria of the slaughtered birds in response to increased slaughter age are shown in

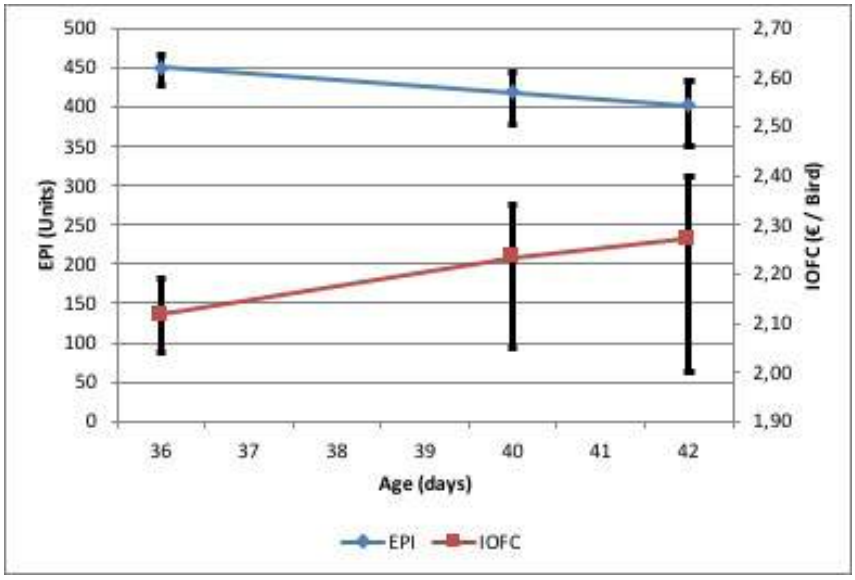

Figure 2: Means (across all lines) and SD of the European Production Index (EPI) and Income Over Feed Cost (IOFC) from day 36 to 42

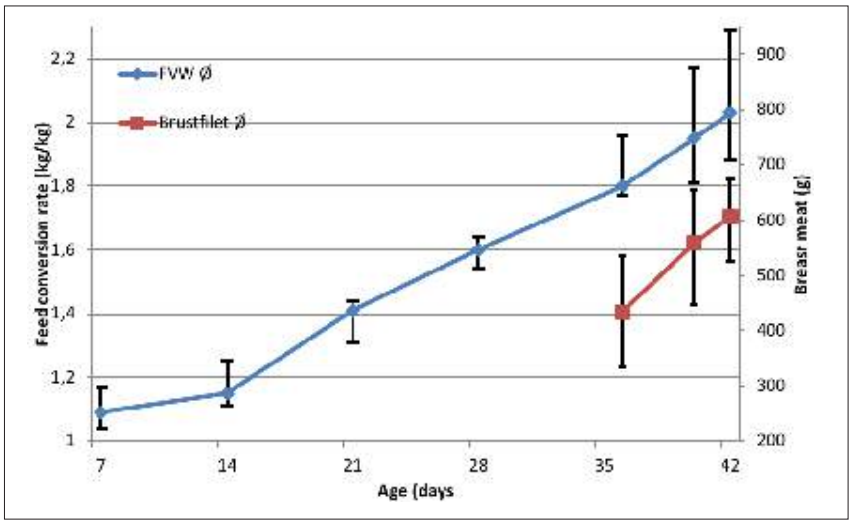

Figure 3: Development of breast meat (g) from 36 to 42 days and feed conversion rate from 7 to 42 days of age

table 5. Mean slaughter weight increased in response to slaughter age from 2080 to 2409 and 2532 g. The difference between minimum and maximum values decreased with slaughter age. Slaughter yield increased with slaughter age from 68.6 to 70.6 %. The improvement in slaughter yield was mainly supported by the increase of the valuable cuts. From slaughter age 36 to 42 days the weight of breast with skin increased from 435 (20.7%) to 607 g (23.8 %). Here again there exist extremely high differences between the minimum and maximum values, from 16.6 to 24.5 % at 36 days and 20.7 to 26.6 % at 42 days. The breast meat yield from day 36 to 42 is plotted against the feed conversion rate in figure 3. The increase of breast meat yield from day 36 to 42 is higher than the decrease in overall feed conversion. This leads to an improvement of the feed efficiency related to breast meat yield. 12.7 kg of feed is required to produce one kg of breast meat at day 36. This ratio is reduced to 12.0 kg at day 42. Breast meat is of particular importance as the demand for this valuable part is increasing among the consumers in Europe. As shown in figure 3 the development of the breast muscle starts relatively late in the growing period. It is therefore advised to consider this when formulating the finisher diet. In contrast to broiler, the level of crude protein in the finisher diet of ducks has to be increased at the end of the growing period so as to allow optimum growth of breast meat.

The percentage of other parts did not show great changes with increasing slaughter age. There are tendencies of relative decrease for thigh, liver, heart and gizzard.

Conclusions

The present data provide information on the state of productivity of the actual commercial duck breeds. The results of a performance test of 5 different breeds show a considerable progress in body weight gain, feed conversion rate, slaughter yield and breast meat yield, when compared with data from earlier tests. There exists a high variation among breeds. Consequently the most economic age of slaughter may vary depending on growth rate and breast meat yield. Under practical conditions other aspects, such as feather cover and its influence of plucking, health and mortality have also to be considered.

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