



Development of myopathies in broiler chickens with a special insight into spaghetti meat

Reporting

Project Information

DEMyo

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Investment in EU policy priorities

Digital agenda	<input type="radio"/>	Clean air	<input type="radio"/>
Artificial Intelligence	<input type="radio"/>	Climate action	<input type="radio"/>
Biodiversity	<input type="radio"/>		

Coordinated by

UNIVERSITA DEGLI STUDI DI PADOVA



Periodic Reporting for period 1 - DEMyo (Development of myopathies in broiler chickens with a special insight into spaghetti meat)

Summary of the context and overall objectives of the project



To meet increasing demands for high-value proteins, broiler selection programs driven by growth rate led to the development of wooden breast (WB), white striping (WS), and emerging and underexplored myopathy such as spaghetti meat (SM). Although pectoralis major myopathies do not threaten safety, they impair meat quality, challenging the nutritional value of the breast meat, meat quality, and sensory acceptability, and causing enormous economic losses to the poultry industry.

In light of the current situation, the DEMyo project tried to address important questions in myopathies research: What is the difference among animals developing WS, WB, and SM? How can we understand the molecular mechanism involved in developing broiler myopathies in both sexes? And importantly, can the incidence of these myopathies be reduced through dietary and management interventions?

The primary objective of the DEMyo project was to apply an OMICS approach to study the development of SM, WS, and WB in fast-growing broiler chickens of both sexes. Additionally, the project aimed to assess whether nutritional strategies, including the use of sodium butyrate and sustainable protein sources like *Chlorella vulgaris*, could reduce the occurrence of myopathies, particularly under challenging conditions such as heat stress that encourage the development of myopathies.

Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far



Work within the DeMYO project was conducted via 6 work packages (WPs).

WP1 comprised two in vivo experimental trials: one where the diet of 792 (half male and half female) broiler chickens was modulated through supplementation with microencapsulated sodium butyrate (Na-butyrate; 0, 150, and 300 mg Na-butyrate/kg diet) and dietary fat reduction (7.7% vs. 6.7% in the grower diet; 8.9% vs. 7.7% in the finisher diet); other was performed on 576 Ross 308 chickens housed for 42 days in 36 pens in 2 rooms of a poultry house, according to a full factorial design encompassing 2 room temperatures (standard vs. high), 2 sexes (females vs. males), and 3 dietary treatments, that is, diet C0 (control diet), diet C3, and diet C6 containing 0, 3, and 6%, respectively, of *C. vulgaris* biomass replacing the same quantities of soybean meal. In both trials, animal growth performances were recorded, whereas, postmortem, myopathies occurrence were recorded, the jejunum was sampled for histological analysis and caecal content to determine microbiome, and finally breast meat was collected to evaluate for quality, texture, chemical, fatty acid, amino acid and mineral composition, oxidation and sensory attributes. In the second trial, additionally, normal and P. major muscles affected with WB, WS, and SM were collected from broilers fed a control diet in both male and female animals for transcriptomic and histological analysis. All laboratory work and sample analysis were managed under WP2.

In WP3, for researcher training, the fellow receives specific training in RNA and DNA extraction, transcriptomic, bioinformatics, and NGS analysis by activity in the labs of DAFNAE and BCA. As for

broiler myopathies, meat quality, and poultry production techniques, the candidate followed the teaching activities of the supervisor within the Master of Agricultural Sciences and Technologies at UNIPD, joined the University of Bologna for a 2-months secondment and the lab at Universitat Politècnica de València (UPV), Animal Science and Technology University Research Institute. The fellow also received training on “Big data and digital tools applied to livestock production” organized by UNIPD, “Science Communication”, “Transferrable Skills: From Research to the Market Outside Academia”, “Transfer in Sustainability: Circular Economy Approaches”, and “Social Innovation” of Arqus Pilot Training Programme “Arqus Transfer Skills”, hosted by Vilnius University.

To transfer knowledge, she conducted three seminars, two within the teaching context of the courses held for the UNIPD students of the Master/Bachelor courses of the AVM School at UNIPD and one for the students of the PhD course at UPV. She provided supervision and mentoring for early career researchers. Fellow supervised two master students and was involved in two PhD theses at DAFNAE. She was one of the tutors in the program “Precision Tools for Sustainable and Welfare-Friendly Animal Farming” organized by UNIPD (Italy), VetAgro Sup (France), Universitat Politècnica de Valencia (Spain) and Université KU-Leuven (Belgium).

To ensure the maximum impact of the results dissemination activities were managed by the specific WP4. Results of this MSCA are reported in 7 journal publications: (1) published paper on dietary supplementation with *Chlorella vulgaris* in broiler chickens submitted to heat-stress: effects on growth performance, meat quality and myopathy occurrence; (2) published paper on the effect of dietary fat content and supplementation with sodium butyrate on growth performance, carcass traits, meat quality, and myopathies in broiler chickens; (3) forthcoming papers on comparative transcriptome analysis of p. major muscles affected by white striping, wooden breast and spaghetti meat in male and female broilers; (4) forthcoming papers on histological findings and differences between p. major muscles affected by white striping, wooden breast and spaghetti meat in male and female broilers; (5) forthcoming papers on nutritional composition, technological quality, and sensory attributes of chicken breast meat affected by WS, WB and SM; (6) forthcoming papers on effect of *Chlorella vulgaris* on gene expression profiles of the small intestine, intestinal microbiota and morphology in heat stressed broiler chickens; (7) forthcoming papers on dietary supplementation with sodium butyrate: gut response at different ages and microbiota composition of female and male broiler chickens. The results from DEMyo have been presented at five international conferences: four via poster communication and four talks, of which one was recorded, and the video is available on the project website. The results from the present project have been included in one master thesis (published in 2023) and two PhD thesis (ongoing).

With WP5, DEMyo's communication strategy employed different channels, including the website (<https://demyo-project.eu/>) and social media, information materials, conferences, workshops, and other events relevant to the project topic to inform the public and communicate the project objectives, results, and increase the visibility of the project. The project was managed under WP6.

Progress beyond the state of the art and expected potential impact (including the socio-economic impact and the wider societal implications of the project so far)



As for growth performances, whereas Na-butyrate supplementation did not affect growth, the reduction in dietary fat moderately impaired growth performance. The effect of dietary *C. vulgaris* inclusion was dose-dependent, and while the addition of 3% of microalgae did not influence production traits, the 6% impaired growth and body weight without changing the feed conversion ratio. Sex dimorphism was evident, with males displaying higher feed intake and growth and more favorable feed conversion than females. Further, exposure of chickens to chronic heat stress impaired growth performances.

As for meat quality and nutritional value, dietary supplementation with Na-butyrate and fat reduction did not affect meat quality, while dietary *C. vulgaris* biomass inclusion resulted in higher redness and yellowness of the breast muscle and a higher proportion of n3 polyunsaturated fatty acids (PUFA). Regarding myopathies, among 180 carcasses, 25.55% had WS, 8.89% had WB, and 18.33% exhibited SM. Dietary manipulations with Na-butyrate and *C. vulgaris* did not affect the myopathy rates. However, fat reduction in diet of approx 1% reduced the occurrence of WS (-38%; $P < 0.01$) WB (-48%; $P < 0.05$) and SM (-90%; $P < 0.01$).

. WB had a major impact on meat quality and resulted in discoloration, higher cooking losses, harder texture, and significant alterations in fatty acid composition, with higher levels of n3 PUFA, n6 PUFA, and total PUFA compared to normal and SM meat.

Transcriptome data showed that the breast transcriptional profile of broilers with WS, WB, and SM was significantly different from that of normal broilers. The impact of SM was extraordinarily high; indeed, this myopathy resulted in 4,553 DEGs (2,282 upregulated and 2,271 downregulated genes), whereas in the broilers with WB, moderate gene expression changes were observed: a total of 386 DEGs were found (330 upregulated and 56 downregulated genes). Weak transcriptional differences were observed in the WS group, showing 8 DEGs only (5 upregulated and 3 downregulated genes). Looking at the downregulated genes in broilers with SM, a total of 4 GO terms and 15 KEGG pathways were significantly over-represented; as concerns the upregulated genes, 20 GO terms and 18 KEGG pathways were enriched. The functional analysis demonstrated that several downregulated genes play a role in muscle contraction, amino acids, and glucose metabolism; while upregulated genes are mostly involved in cell signaling and differentiation, and immune processes.

Histological analysis revealed a reduced percentage of normal fibers and an increased rate of degenerative and necrotic fibers in myopathic muscles compared to normal muscles. Muscles with myopathies were characterized by inflammatory cell infiltration, more collagen, and adipocytes than normal muscles. No significant differences in jejunal morphology were observed due to dietary interventions, although villi height and crypt depth increased with age. Heat stress led to shorter villi, and males exhibited a higher villus-to-crypt ratio than females. Microbiota analysis identified Firmicutes as the dominant Phylum with sex-related differences in microbial diversity. While alpha diversity remained unaffected by diet, sex, and temperature stress, beta diversity showed significant temperature-related differences in microbial community structure.



ig-3-breasts-with-spaguetti-meat.jpg



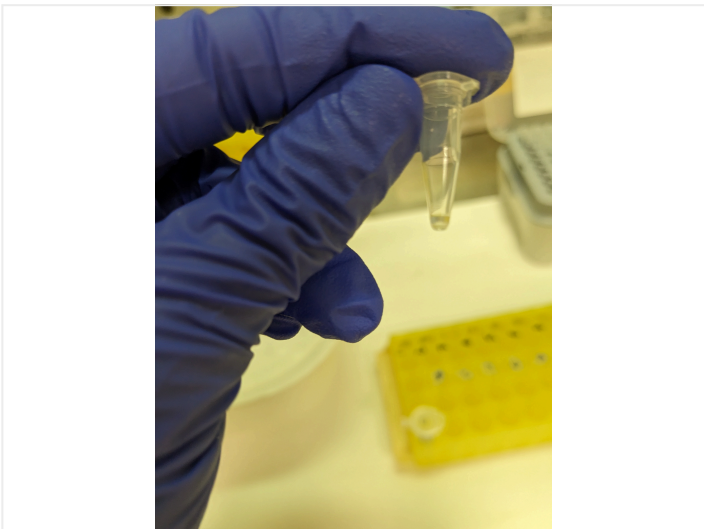
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ig1-marija-chickens-farm.jpg

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