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IDENTIFICATION OF FARMING PRACTICES THAT MAY INFLUENCE ANTIOXIDANTS IN CHICKEN BREAST AND THIGH MEAT

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I. INTRODUCTION

Oxidative stability of chicken meat is influenced by farming practices, which vary in genetics, nutrition, and management across a broad array of production systems [1]. A well-functioning antioxidant defense system is crucial in delaying oxidative changes of chicken meat during processing and storage [2]. Among the key antioxidants in meat, α-tocopherol is a lipid-soluble antioxidant that mitigates oxidative stress and supports immune function [3]. Additionally, the histidine-containing dipeptides (HCDs) carnosine and anserine contribute to the antioxidant defense in muscle tissue [4]. Beyond their antioxidant properties, these compounds may also offer other health benefits to meat consumers. This study aimed to determine which farming practices across production systems can effectively affect antioxidant concentrations in chicken breast and thigh meat.

II. MATERIALS AND METHODS

A total of 89 chicken farms belonging to diverse production systems were chosen from five European countries involved in the H2020 INTAQT project: Belgium (n = 20), France (n = 26), Italy (n = 25), Poland (n = 10), and Switzerland (n = 8). Farming practices (n = 19) were selected from recorded questionnaires which included categorical variables such as genotype, litter type, heating system, and ventilation system, as well as numerical variables such as age at slaughter (age), live body weight (LBW) at slaughter, average daily weight gain (ADG), stocking density, outdoor duration, outdoor area, and litter quantity. Feed composition (finisher phase diet) variables included protein, fat, fiber, and ash content (in %), as well as dietary levels of vitamin E, vitamin A, and vitamin D. After slaughtering, 20 carcasses per farm were randomly selected. On the second day post-slaughter, breast and thigh meat were excised, minced, and pooled separately per farm, resulting in 89 thigh and 89 breast composite samples. All composite samples were analyzed in duplicate for α-tocopherol, carnosine, and anserine. A Random Forest (RF) model was used to investigate the relationships between farming practices (predictors) and the levels of the antioxidants α-tocopherol, carnosine and anserine in meat (response variables). In this model, we implemented bootstrap resampling (50 iterations), where each RF model consisting of 500 trees (*num.trees* = 500) was trained on different resampled datasets, with 4 predictors (*mtry* = 4) randomly selected at each split. Briefly, in each iteration, 67% of the data was randomly sampled with replacement to create the bootstrap training data set. The remaining 33% of the data was used as the Out-of-Bag (OOB) data for internal validation of the RF model. *RF function* and *importance function* in R were applied to build separate models for each response variable, to quantify the contribution of each predictor using the mean decrease in accuracy (MDA) score. This score represents the reduction in mean accuracy of response variable when a specific predictor is randomly shuffled. MDA is expressed in the same unit as the response variable, enabling better generalization and interpretation. Model performance was evaluated on OOB data using mean squared error (MSE) and R-squared (R^2), with lower and higher values reflecting better performance respectively. A Conditional Inference Tree (CIT) was fitted using *ctree (partykit)* with the top 5 predictors (based on MDA scores) to interpret their direction of association. CIT split the data using significance tests ($P < 0.05$), selecting the most relevant variables while minimizing bias.

III. RESULTS AND DISCUSSION

In this study we used RF modeling to identify the most important predictors as it is non-parametric, robust to multicollinearity, and capable of handling both numerical and categorical variables. It can also be helpful in ranking

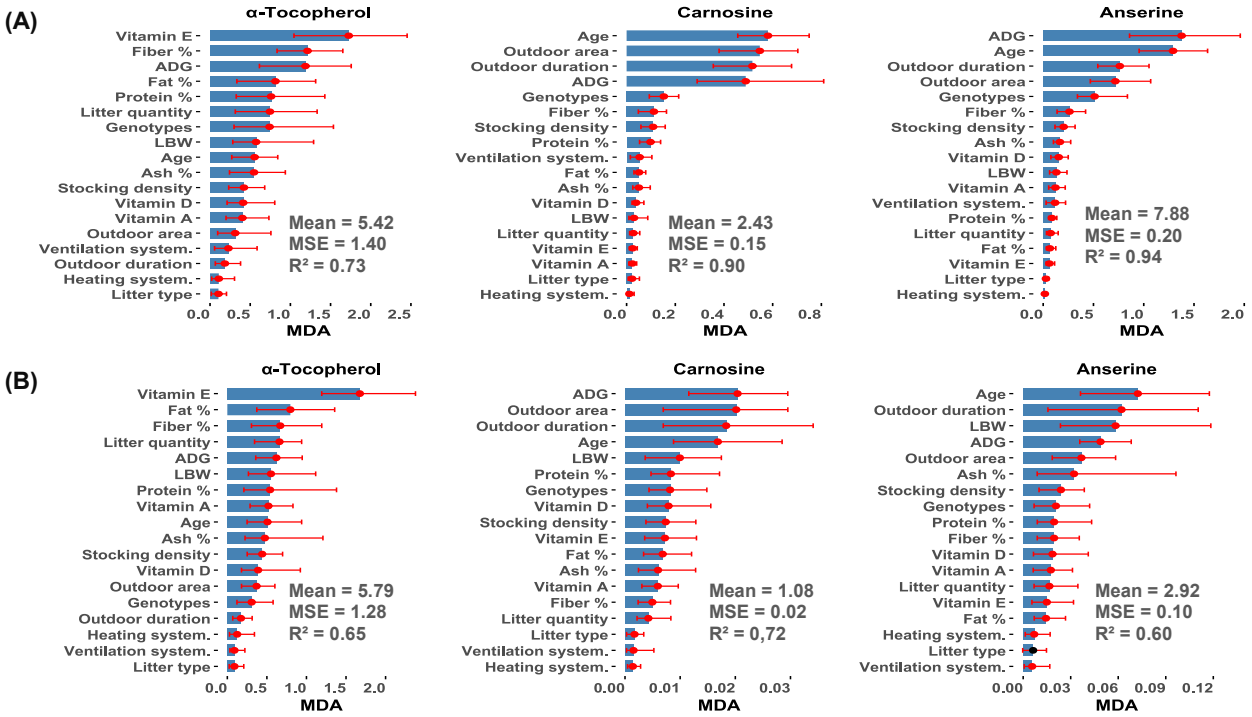


Figure 1. Variable importance plots from Random Forest analysis for breast (A) and thigh (B) chicken meat

the farming practices according to their importance. In breast meat (Figure 1 A), based on the MDA score, the vitamin E level in feed was the most influential practice in predicting α-tocopherol levels, followed by fiber level in feed and ADG. For breast carnosine and anserine, age, ADG, outdoor area and outdoor duration were the most influential practices with high MDA scores. RF predictions were excellent ($R^2 \geq 0.80$) for carnosine and anserine and very good ($0.70 \leq R^2 < 0.80$) for α-tocopherol. In thigh meat (Figure 1 B), vitamin E in feed was the most important variable in predicting α-tocopherol. ADG, outdoor area, outdoor duration and age were most important for thigh carnosine prediction. For thigh anserine, age had the highest MDA score followed by outdoor duration, LBW and ADG. RF predictions were good ($0.60 \leq R^2 < 0.70$) for α-tocopherol, carnosine and anserine. CIT showed that vitamin E in feed and ADG were positively associated ($P < 0.05$) with α-tocopherol levels in breast and thigh meat. ADG was negatively associated while age was positively associated ($P < 0.05$) with breast carnosine and anserine. In thigh meat, outdoor duration and LBW were positively associated ($P < 0.05$) with carnosine, whereas age was positively associated ($P < 0.05$) with anserine.

IV. CONCLUSION

Among all practices, the feed vitamin E concentration was the most important factor in influencing the α-tocopherol level in chicken meat [5]. HCDs in breast and thigh meat were influenced by a combination of factors, linked to the farm system model; as growth rate (slaughter age and ADG) and outdoor rearing conditions (outdoor area and duration). However, these findings should be validated using larger and more complex datasets, as the order of importance may change with the inclusion of additional data and farming practices in the analysis.

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