Cyprus University of Technology

Department of Agricultural Sciences, Biotechnology and Food Science

Doctoral Dissertation

Feeding By-product Feedstuffs Affected Milk Fatty Acid Profile and the Expression of Lipogenic Genes in Ruminants

Marina C. Neofytou

Limassol, April 2021

CYPRUS UNIVERSITY OF TECHNOLOGY

FACULTY OF GEOTECHNICAL SCIENCES AND ENVIRONMENTAL MANAGEMENT

DEPARTMENT OF AGRICULTURAL SCIENCES, BIOTECHNOLOGY AND FOOD SCIENCE

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Approval Form

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ABSTRACT

The present thesis aimed to evaluate industrial by-products, ensiled olive cake (OC), and dried distillers' grains with solubles (DDGS) as diet components to ruminants. Since nutrition is the primary environmental factor regulating milk's concentration and composition, particularly milk fat, feeding industrial by-products rich in unsaturated fatty acids (FA), like DDGS and OC, is a reasonable attempt to improve the nutritional quality of milk and dairy products.

In this regard, the effect of dietary inclusion of 10% (on diet DM) of ensiled OC, as a replacement of forages, was evaluated in two experiments, investigating the effects on milk yield, composition and FA profile of cow milk (**Chapter 2 and 3**) and related Halloumi cheese (**Chapter 3**). Moreover, for examining any possible impact of this oil rich by-product feeding on bovine lipid metabolism, changes in the expression of selected genes involved in mammary and adipose lipid metabolism were assessed (**Chapter 3**). The results of these studies showed that the inclusion of 10% (DM) of ensiled OC did not affect milk yield and improved the quality of milk and Halloumi cheese by reducing saturated lipids and increased unsaturated ones including oleic (C18:1 *cis*-9), vaccenic (C18:1 *trans*-11) and conjugated linoleic (CLA *cis*-9, *trans*-11; rumenic acid) acids, all related to positive effects to human health. Milk FA differences observed were not associated with alterations in the mammary expression of genes involved in FA synthesis, uptake, translocation, and lipogenesis regulation. However, an upregulation of *SREBF1* mRNA expression in perirenal fat of cows fed the OC diet was demonstrated.

Furthermore, to evaluate the use of this by-product on other ruminant species, an additional study was implemented in lactating goats testing the effects of 10 and 20% inclusion rates (DM) of ensiled OC supplementation (OC10 and OC20 groups,

respectively) on milk yield, composition and FA profile (**Chapter 4**). The expression of genes related to mammary and adipose lipid metabolism of Control and OC20 groups was also evaluated for examining the effect of OC feeding on mammary and adipose tissue metabolism. The results of this study confirmed those observed in cows. More specifically, all major saturated FA between C4:0 to C16:0 were reduced, and consequently, the atherogenic index was diminished, while the content of long-chain (>16 carbons; LCFA) and mono-unsaturated FA (MUFA) were enhanced in the caprine milk of both OC groups. Among individual UFA, increments of oleic, C18:1 *trans*-10, and vaccenic acids were demonstrated in both OC groups, as well as in the concentration of rumenic acid, which was increased by 11 and 21% ($P < 0.001$) with OC10 and OC20 diets, accordingly, compared to Control. Additionally, the supplementation of goat diets with 20% (DM) of ensiled OC altered the mammary expression of *SLC2A1* ($P < 0.05$), *VLDLR* ($P < 0.01$), *FABP3* ($P < 0.01$) and elevated the *SLC2A1* ($P < 0.05$) and *FASN* ($P < 0.01$) genes in the adipose tissue.

Finally, another by-product that was also studied was the wheat based-DDGS included at rates of 6% and 12% (DM) in diets of dairy ewes, as a replacement of concentrate mix, investigating its potential effects on milk production and content, as well as on milk FA composition (**Chapter 5**). The results showed that milk yield, milk protein percentage, protein, and fat yield did not differ between groups. In contrast, milk fat percentage decreased only in the group contained the highest inclusion rate of DDGS compared to the Control group. The milk FA profile of ewes fed both DDGS diets improved significantly. In particular, the concentration of total SFA was reduced, while long-chain, mono-unsaturated, and poly-unsaturated FA content was increased in the milk of DDGS groups. Additionally, the content of linoleic (C18:2n-6) and rumenic acids was

elevated in both DDGS groups compared to control. Changes in FA profile resulted in a decline in the atherogenic index of milk by 20% and 35% in the groups contained 6 and 12% (DM) of DDGS, respectively, compared to control.

Overall, the findings of the present dissertation, which are summarized and discussed in **Chapter 6**, are very promising and fully support the use of these by-products (OC and DDGS) in ruminant diets for improving the quality of milk and derived Halloumi cheese, opening new insights and perspectives for their practical application and future research.

Keywords: *olive cake**,** wheat distillers' grains with solubles**,** rumenic acid, fatty acids, gene expression, dairy cows, ewes, goats, Halloumi cheese, milk production*