PhD THESIS

Development of optimal nutritional strategies to improve the quality of milk in Jersey cows

(SUMMARY OF Ph.D. THESIS)

PhD student **Anita Șánta**

Scientific coordinator **Prof. dr. Daniel Mierliță-Pantea**

ABSTRACT OF Ph.D. THESIS

The doctoral thesis entitled **"Development of optimal nutritional strategies to improve the quality of milk in Jersey cows"** is structured in two main parts: the bibliographic documentation and the personal contributions. The doctoral thesis includes a number of 8 chapters and the bibliographic references used. The full work consist in a total number of 157 pages, 26 tables, 49 figures and 132 bibliographic references.

Part I of the thesis - **The current state of knowledge** - includes two chapters, in which are presented the newest knowledges in the field of doctoral thesis, respectively: Chapter 1 "The milk content in nutrients and bioactive compounds with implications in human health" and Chapter 2 "The feeding influence on the milk composition and the content in bioactive compounds". The first part includes 20 pages, which represents 12.74 % of the total thesis volume.

Part II of the thesis - **Personal contributions** - includes five chapters: Chapter 3 "*Aim, objectives and research organization*"; Chapter 4 "*Materials and methods of the research*"; Chapter 5 "*The influence of feeding type which combining the grazing with TMR (total mixed diet) on productive performances and milk quality*"; Chapter 6 "*The influence of the concentrates proportion and forage diet supplementation with rapeseed on productive performances and milk quality*". In chapter 7 are presented the *general conclusions and recommendations*, and in the chapter 8 are presented *the originality aspects and innovative contributions* of this doctoral thesis.

Part II includes a number of 98 pages, which represents 62.42% from the total thesis volume, 23 tables and 49 figures.

Milk fats contain high amounts of saturated fatty acids (SFA) and low amounts of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), being often criticized for the negative impact on the consumers health. SFA predispose to the occurrence of cardiovascular diseases and coronary problems. On the contrary, unsaturated fatty acids (UFA) and especially polyunsaturated ones (PUFA) prevent against the occurrence of cardiovascular diseases, obesity, diabetes and cancer without requiring major changes in consumers eating habit. On the other hand, increasing the concentration of nutritionally beneficial unsaturated FAs will increase the risk of milk fat oxidation, modifying the nutritional and dietary properties of milk. Therefore, to maintain a high milk quality, the concentration of antioxidants should be increased, thus will increase the oxidative stability of milk.

The available field literature highlight a limited number of researches, but also heterogeneous results regarding to the influence of feeding type on the FA profile of milk fats, on the content of lipophilic antioxidants and total antioxidant capacity (TAC) of milk. Consequently, **the aim of this research was to evaluate the effects of different feeding types on the milk production and composition, fatty acid profile and antioxidant status of milk resulted from Jersey cows**. It is important to determine if by using optimal nutritional solutions the content of milk can be improved in those fatty acids considered beneficial for human health (especially FA n-3, VA and CLA) and if the oxidation risk is compensated by the higher concentrations in lipophilic antioxidants (mainly α-tocopherol, retinol and β-carotene) existing in some feeds and

transferred to milk. In order to achieve this aim, the conducted researches had two main objectives.

The first major objective was to determine the effect of using pasture in combination with a total mixed ration (diet) (pTMR) on the milk production and composition, fatty acid profile, fat-soluble antioxidants content and total antioxidant capacity (TAC) of milk. The secondary objective was to ***evaluate the changes in the FA profile and lipophilic antioxidants from milk during the grazing period in relation to the chemical composition of the pasture.*** In addition, ***the influence of milk pasteurization and storage on the lipophilic antioxidants content and oxidative stabilities of milk was studied.*** Within this objective, the following hypotheses were tested:

(1) the use of grazing during the day period (8 hours/day) causes important changes in the chemical composition, FA profile, non-enzymatic lipophilic antioxidants content and in the total antioxidant capacity (TCA) of fresh milk, after pasteurization and storage; **(2)** the nutritional and sanogenic quality of the milk obtained in the pTMR system (pasture for 8 hours/day + TMR) are dependent by the periodic variations in the pasture chemical composition.

Sixteen Jersey dairy cows in the second half of lactation were divided into two homogeneous groups and randomly assigned to the two different feeding systems: total mixed ration (TMR) or partial mixed ration (pTMR: grazing 8 h/day + TMR) . The same mixt TMR was used in both treatments, being ad libitum indoors. The experimental period for the cows from pTMR group, was divided into three grazing periods (sampling periods - P) of 3 weeks each: P1 (May: 10-30 May), P2 (June: 31 May – June 20) and P3 (June/July: June 21 – July 11).

Among the established laboratory methods, the chemical composition, fatty acids and lipophilic antioxidants content were performed for raw materials used for TMR mixt, for TMR rations and the pasture corresponding for those three grazing phases. For the evaluation of productive performances were determined: the body weight of the cows at the beginning and at the end of the experimental period, the evolution of milk production, the feed consumption and the degree of feed valorization and the economic efficiency of the tested nutritional variants. The milk quality was assessed based on the content in nutrients (protein, fat, lactose), urea and somatic cells. The quality of milk fats was assessed by analyzing the fatty acids profile and calculating the sanogenic lipidic indices of milk fat (atherogenic index - AI, thrombogenic index - TI, health promoting index - HPI, PUFA/SFA ratio, omega-6/omega -3 FA and hypo- / Hypercholesterolemic FA). The antioxidant status of milk was evaluated from raw, pasteurized and and stored for 4 days at 2-4˚C (refrigerated) by determining the content of non-enzymatic lipophilic antioxidants and total antioxidant capacity (TAC).

The **second major objective** was to **evaluate the effects of the two FC ratios (forage:concentrate) and feed supplementing with milled rapeseed (MR) on the milk production and composition, fatty acids profile and antioxidant status of milk resulted from Jersey cows.** In addition, ***the influence of milk pasteurization and storage on the content of lipophilic antioxidants and oxidative stability of milk was studied.*** Within this objective, the following hypotheses were tested: **(1)** the concentrates percentage in the cows' feed but also the direct interaction between the FC ratio and rapeseed will determine important changes in the fatty acid profile of milk fats and in the antioxidant status (the antioxidants content and total

antioxidant capacity) of milk; **(2)** the nutritional and sanogenic quality of milk can be improved by supplementing the feed with milled rapeseed.

A complete 2 x 2 factorial arrangement was organized, using the method of 4 x 4 Latin square: 4 periods of 4 weeks each, 4 groups of 4 cows/group and 4 treatments (diets). Dietary treatments consisted in total mixed rations (TMR) iso-nitrogenous, with the concentrate (FC) ratio at a high (65:35 - LC) or low (50:50 - HC), supplemented with milled rapeseed (MR) (LR and HR), or without MR supplement (L and H). The total mixed rations (TMR) consisted in grass silage, alfalfa hay (in a ratio of 2:1, reported at DM) and an concentrates mixture, which were supplemented with milled rapeseed at a dose of 0 (L and H, respectively) or 64 g/kg TMR (LR and HR). The amount of rapeseed was calculated to provide an additional of 30 g oil/kg DM (approx. 540 - 600 g rape oil/day/cow). Each experimental period consisted on 28 days, of which the first 21 days were for adaptation to the new diet, and in the last 7 days the productive performances were recorded and samples were collect for the laboratory analyses.

In order to achieve the proposed objectives, the same aspects as those mentioned in the previous objective were followed during this research: the chemical composition, the fatty acid profile and the content of lipophilic antioxidants of the tested TMR ingredients and feeds; evaluation of productive and economic performances; evaluation of some milk quality indicators; the evaluation of some quality indicators of milk fats and the evaluation of the antioxidant status of raw, pasteurized and stored milk.

The raw data obtained in the trial of productive effects and from the laboratory analyzes were statistically processed as repeated measures over time using SAS software (Statistical Analysis Software, version 9.1 Inst. Inc.). The statistical significance of the results was tested by ANOVA (Analysis of Variance) with repeated measurements, with the factorial factor for diet type. The statistical model included the diets (experimental treatments) as fixed effects, and the data obtained in the pre-experimental period were included as covariates in the statistical model. The experimental periods (grazing periods and sampling periods respectively) were considered as repeated factors, and cows were the experimental units. Multiple comparisons between means were performed by Tukey's method.

Based on the obtained results in the research carried out for the development of some optimal nutritional strategies in order to improve the nutritional quality of milk in Jersey cows, the following conclusions were formulated:

(1) Conclusions regarding the influence of the diet type which combine the grazing with TMR on the milk production and quality.

- Compared to the grass from pasture, the TMR ration had a higher content in dry matter (DM), crude protein and non-nitrogenous extractives, but a lower level in crude fibre. The chemical composition of the pastures changed during the experimental period; in the first grazing period (P1), the grass had the lowest content in dry matter and crude fibre but the highest crude protein content (N x 6.25). The forages crude fat content did not show significant differences between TMR and pasture, and also not between the three grazing periods.
- The pasture contain higher proportions of polyunsaturated fatty acids and especially α-linolenic acid (C18:3 n-3), while the total mixed ration (TMR) contain

a higher proportion in linoleic acid (C18:2 n-6) and oleic acid (C18:1 cis-9). The content in α-linolenic acid (C18:3 n-3) decreased with the development of the plants (P1 <P2 <P3), demonstrating that between the pasture content in crude protein and α-linolenic acid (C18:3 n-3) there is a strong positive linear relationship.

- Comparativ cu dieta TMR, conţinutul păşunii în β-caroteni a fost de 10 ori mai mare (6,73 vs. 66,97 mg/kg SU), iar cel de α-tocoferol a fost de 3-4 ori mai mare (8,64 vs. 20,1-34,4 mg/kg SU); nivelul antioxidanţilor din păşune scăzând treptat odată cu înaintarea plantelor în vegetaţie.
- Compared to the TMR diet, the pasture content in β-carotene was ten times higher (6.73 vs. 66.97 mg/kg DM), and that of α-tocopherols was 3-4 times higher (8.64 vs. 20.1-34.4 mg/kg DM); the antioxidants level in the pasture gradually decreasing with the plants development in vegetation.
- Milk production was significantly lower at cows from the pTMR group compared to cows from the TMR group, due to the significantly lower energy content in the pasture compared to TMR ration (1.44 vs. 1.66 Mcal ENL/kg DM). No significant differences were found between the treatments regarding the corrected milk production (FCM: fat-corrected milk and ECM: energy-corrected milk). The lower milk production in the P2 and P3 grazing period is consistent with the lower content in ENL and crude protein and with the higher crude fibre content of the pasture grass, compared to the P1 period.
- The TMR consumption in cows of pTMR group which had access to pasture decreased on average by 4.31 kg DM/head/day compared to TMR group cows (18.11 vs. 13.80 kg DM/day) ($p < 0.001$). Also, in the case of pTMR group of cows, the pasture intake (kg DM/day) decreased with the development of plants in vegetation (P1 >P2 >P3) due to the gradual increase crude fibers content in plants.
- The consumption of TMR and concentrates per liter of milk was lower in cows which had access to pasture, which indicates an improvement in the use of feed through the efficient and sustainable usage of a local and cheap forage resource. The better quality of the pasture in the first grazing period (P1) determined a reduction in the concentrates consumption and respectively in TMR/l of milk, compared to the P2 and P3 grazing periods.
- Feed costs, related to production of one milk liter (physical milk or milk corrected for fat content - FCM or energy content - ECM), were lower by up to 17.64% in the case of the pTMR group of cows, which means that by combining the grazing with the TMR ration in the nutrition of dairy cows is the most beneficial from the economic point of view.
- The milk obtained from the cows of pTMR group had a significantly higher content in fat, urea and somatic cells and lower in lactose. No significant differences were found between the treatments regarding the total fat, protein and lactose production. The protein and lactose content in milk did not change significantly

during on that three grazing periods, while the fat content increased (4.78%-5.21%), and the urea and somatic cell content decreased ($p < 0.05$).

- Milk produced by the cows which benefited by limited access to pasture (pTMR diet) had an improved fatty acids profile, with a higher concentrations in FA considered beneficial for human health (VA, CLA, omega-3 FA) and a lower concentrations in FA with hypercholesterolemic effect (C12:0, C14:0 and C16:0). This changes in the FA profile was correlated with the improvement in sanogenic lipidic indices of milk fat (n-6/n-3 FA, hypocholesterolemic/hypercholesterolemic -h/H, atherogenic index -AI, thrombogenic index -TI and health promotion index -HPI). The milk obtained during the P1 grazing period had a higher concentrations in VA, CLA and n-3 FA and lower in C14:0 and C16:0, recording the best values for the main sanogenic lipidic indices of fat. By comparison, cows fed with the TMR diet produced more milk but with lower fat and protein content, and the milk had a higher content in SFA and n-6 FA (mainly C18:2 n-6).
- The milk of cows which grazed for 8 hours/day (pTMR group) had a higher concentrations of α-tocopherol, retinol and β-carotene, which were positively correlated with the total antioxidant capacity (TAC) of milk. The TAC value was higher in the pTMR group for both raw and pasteurized milk, respectively for pasteurized and stored in fridge for 4 days, compared to the TMR group. Thermal treatment did not affect the milk content in α-tocopherol and retinol, but keeping pasteurized milk for 4 days in the frigde had a negative effect on the concentration of these natural antioxidants. During storage, the antioxidant activity does not change significantly, therefore the consumers could benefit by these nutritional qualities and bioactive compounds of milk during the 4 days of keeping pasteurized milk in the fridge.

(2) Conclusions regarding the influence of the concentrates proportion and the supplementation of feed ration with rapeseed on the milk production and quality

- The concentrates proportion, respectively the FC ratio (forage:concentrates) of the feed mainly influenced the DM intake, milk production and composition, while the diets supplementation with milled rapeseed (MR) has influenced the fatty acids profile of milk fats and the antioxidant status of milk, respectively its α-tocopherol content and total antioxidant capacity (TAC).
- Increasing the concentrates proportion in the dairy cows feed from 35% to 50% (% of DM of TMR) led to an increase in starch content and a decrease in NDF and ADF content, while the supplementation with milled rapeseed (MR) has doubled the fat concentration in the feed, determining an increase in the energy content (ENL - Mcal/kg DM) of the experimental diets.
- The analysis of the fatty acid composition of fats from the feeds indicated that grass silage contains relatively high proportions of α-linolenic acid (54.27% of the total FA); while oleic acid (C18:1 cis-9) was predominant in rapeseed, and the

concentrate mixture was rich in linoleic acid (C18:2 n-6). Consequently, linolenic acid (C18:3 n-3; ALA) was the predominant fatty acid in the diets with a high FC (LC; 35% concentrate), while the diets with a low FC (HC; 50% concentrate) had a higher content in linoleic acid (LA), and the diets supplemented with MR (LR and HR diets) were more rich in oleic acid (OA).

- The content of α-tocopherol and β-carotenes was higher in grass silage and alfalfa hay, which determined a higher level of these antioxidants in the diets with a high FC. Rapeseeds had a higher α-tocopherol content (47.07 mg/kg DM) compared to the concentrate mixture which had a low antioxidant content.
- Increasing the concentrates ratio in the cows ration lead to the increase of DM and ENL intake (Mcal/day), of milk production and milk protein content, while the milk fat concentration decreased. Rapeseeds did not affect DM intake or milk composition, but increased the milk production only in case of the association with a low FC ratio of the diet. The lactose concentration and DMfg (fat-free DM) in milk were not affected by the experimental treatments.
- The best feed conversion rate was obtained in the case of cows fed with a low concentrate (LC) diets, where the concentrate consumption (g DM/l milk) was lower by up to 38.5% ($p < 0.001$), compared to the case of diets rich in concentrates (HC). Energy efficiency (Mcal/l milk) was not affected by the experimental treatments. In addition, the supplementation of the diets with MR did not affect the concentrates consumption (g/l milk) and the energy efficiency (Mcal/l milk) of the feed.
- Feeding costs related to the obtaining of one liter of milk (physical milk or corrected milk), were higher by up to 10.84% in the case of cows which benefited by forage rations rich in concentrates (HC lots). The including of rapeseed in the feed caused an increase in feed expenses by up to 12.05%; the increases being higher in the case of cows fed with rations with a low concentrates content.
- Decreasing the FC ratio of the diets led to a decrease in the concentration of saturated fatty acids (SFA) and an increase in the polyunsaturated FA (PUFA) in milk fats, while supplementing the diets with MR has reduced ($p < 0.001$) the SFA proportion and increased ($p < 0.001$) the monounsaturated FA (MUFA) level in milk fats. The diets supplemented with MR determined a decrease in the concentration of FA with atherogenic effect and an increase in the level of FA beneficial for the consumers health (C18:1 cis-9, C18:1 trans-11 and C18:3n-3), while the decrease of FC ratio had a negative effect on omega-3 FA.
- The n-6/n-3 FA ratio became more favorable to human health by supplementing the feed with rapeseed. The value of the atherogenic index (AI) and thrombogenic index (TI) has decreased and the ratio of h/H FA increased in the milk of cows fed with diets rich in concentrates (HC), but also when the diets were supplemented with rapeseeds, regardless the FC ratio of the diet (LR and HR diets). The increase of concentrates amount in feed and the diets supplementation with MR improved

the quality of milk fats, this being proved by the increase in the values of the health promoting index (HPI - health promoting index). Therefore, the results obtained in this research confirmed that milk with the highest fat quality was obtained from the cows fed with the diet rich in concentrates and supplemented with MR (diet HR), while the diet with a low concentrates content and without addition of MR (diet L) has determined the lowest quality of milk fats, in terms of the impact on human health.

- The oxidative stability of milk was mainly influenced by the composition of fatty acids and the storage time, as pro-oxidant factors, and by the concentration of tocopherols and carotenoids as antioxidant factors. Alpha-tocopherol, retinol and beta-carotenes showed a higher concentration ($p < 0.05$) in raw milk when the cows' diet had a low concentrates content (LC diets) and the diets supplementation with MR determined an increase ($p < 0.05$) of the α-tocopherol content in milk, while the retinol and beta-carotene showed a downward trend, even if was not statistically suported.
- Increasing the concentrates amount in feed reduced the TAC value, while supplementing the diets with MR lead to improved ($p < 0.05$) the antioxidant capacity of both raw and stored milk for 4 days in the fridge. The highest TAC value was recorded in raw milk obtained from cows fed by diet with a high FC ratio and supplemented with MR. Storing milk for 4 days in a fridge caused a decrease in the concentration of retinol and beta-carotenes in milk, probably due to the reactive oxygen species occured during the storage and which were inactivated by the antioxidants in milk. As a result of this activity, the antioxidants in milk were oxidized, thus leading to a decrease of their concentration in milk, even of α-tocopherol, although this was not statistically suported. Consequently, during storage, the total antioxidant capacity of milk decreased significantly in the case of diets rich in concentrates and did not change significantly in the case of diets with a low concentrates content, being correlated with the antioxidants concentration in milk. Thus, it can be concluded that high levels of α-tocopherol and retinol in milk do not prevent the oxidation of unsaturated FAs but delay this process thus extending the validity period of milk and it by-products. Therefore, the consumers could benefit by the nutritional qualities and bioactive compounds of milk in those 4 days of keeping the milk in the fridge.

The diet type which combines the grazing with TMR given ad libitum in the shelter can be used as a strategy to obtain milk with a higher content of bioactive compounds beneficial for human health (functional fatty acids and lipophilic antioxidants) and with a better oxidative stability. In addition, this strategy for feeding dairy cows allows to saving an important amount of TMR (approx. 4.3 kg DM/day/cow) and the sustainable usage of the available pastures. The concentrations of functional fatty acids (VA, CLA and ALA) and lipophilic antioxidants (α-tocopherol, retinol and β-carotene) could be used as biomarkers to identify the grass-milk, which has a positive effect on the perception and acceptance of milk and milk products by the consumers.

Supplementing the diet with 30 g of oil/kg DM as milled rapeseed is an optimal strategy to increase the fatty acids concentration with a beneficial effect on human health (oleic acid, vaccenic acid, linolenic acid) and to reduce the ratio of fatty acids from milk with an atherogenic effect (especially palmitic acid), which involve a reduction in the risk factors for cardiovascular diseases in humans.

It is important to continue the research in order to establish the effect of these nutritional solutions on the processing and properties of milk products. The increase of unsaturated fatty acids concentration (especially oleic acid, vaccinic acid, linoleic acid, linolenic acid and CLA) in the milk fats could affect the activity of the starter culture in the production of milk products (cheeses, acido-lactic products, butter), as a result of the change in the ratio between the main fatty acids (n-6/n-3; SFA/PUFA; h/H FA; OA/PA; etc).

SELECTIVE REFERENCES

1. AKERT, F.S., KREUZER, M., KUNZ, C., REIDY, B., BERARD, J., 2021, Effects of full-time *vs*. part-time grazing on seasonal changes in milk coagulation properties and fatty acid composition. *J. Dairy Res.,* 88 (1), 23-28, doi.org/10.1017/S0022029921000169.
2. BELIVEAU, A.R., 2012, Variations in carotenoids and retinol in milk and cheese from Jersey cows at an organic dairy compared to a conventional dairy over a pasture season. Master's Theses and Capstones, 743.
3. CHEN, J., LIU, H., 2020, Nutritional Indices for Assessing Fatty Acids: A Mini-Review. *Int. J. Mol. Sci.* 21, 5695. doi.org/10.3390/ijms21165695.
4. COPPA, M., FERLAY, A., BORREANI, G., REVELLO-CHION, A., TABACCO, E., TORNAMBÉ, G., 2015, Effect of phenological stage and proportion of fresh herbage in cow diets on milk fatty acid composition. *Anim Feed Sci Technol*. 208, 66-78. 10.1016/j.anifeedsci.2015.07.006.
5. De La TORRE-SANTOS, S., ROYO, L.J., MARTÍNEZ-FERNÁNDEZ, A., CHOCARRO, C., VICENTE, F., 2020, The Mode of Grass Supply to Dairy Cows Impacts on Fatty Acid and Antioxidant Profile of Milk. *Foods,* 9, 1256. doi.org/10.3390/foods9091256.
6. De La TORRE-SANTOS, S., ROYO, L.J., MARTÍNEZ-FERNÁNDEZ, A., MENÉNDEZ-MIRANDA, M., ROSA-GARCÍA, R., VICENTE, F., 2021, Influence of the Type of Silage in the Dairy Cow Ration, with or without Grazing, on the Fatty Acid and Antioxidant Profiles of Milk. *Dairy,* 2, 716-728. doi.org/10.3390/dairy2040055.
7. KHAN, I.T., BULE, M., ULLAH, R., NADEEM, M., ASIF, S., NIAZ, K., 2019, The antioxidant components of milk and their role in processing, ripening, and storage: Functional food. *Veterinary World*, 12(1), 12-33.
8. KHAN, I.T., NADEEM, M., IMRAN, M., AYAZ, M., AJMAL, M., ELLAHI, M.Y., KHALIQUE, A., 2017, Antioxidant capacity and fatty acids characterization of heat treated cow and buffalo milk. *Lipids Health Dis.,* 16, 163. doi.org/10.1186/s12944-017-0553-z.
9. LERCH, S., FERLAY, A., SHINGFIELD, K.J., MARTIN, B., POMIÈS, D., CHILLIARD, Y., 2012, Rapeseed or linseed supplements in grass-based diets: effects on milk fatty acid composition of Holstein cows over two consecutive lactations. *J. Dairy Sci.* 95, 5221-5241. doi.org/10.3168/jds.2012-5337.

10. MIERLITA, D., SANTA, A., MIERLITA, S., DARABAN, S.V., SUTEU, M., POP, I.M., MINTAS, O.S., MACRI, A.M., 2023, The Effects of Feeding Milled Rapeseed Seeds with Different Forage:Concentrate Ratios in Jersey Dairy Cows on Milk Production, Milk Fatty Acid Composition, and Milk Antioxidant Capacity. *Life*, 13, 46, doi.org/10.3390/life13010046.

11. MIERLIȚĂ, D., 2016, Fatty acid profile and health lipid indices in the raw milk of ewes grazing part-time and hemp seed supplementation of lactating ewes. *S. Afr. J. Anim. Sci.* 46(3), 237-246. 10.4314/sajas.v46i3.3.

12. MORALES-ALMARAZ, E., SOLDADO, A., GONZALEZ, A., MARTINEZ-FERNANDEZ, A., DOMINGUEZ-VARA, A., DE LA ROZA-DELGADO, B., VICENTE, F., 2010, Improving the fatty acid profile of dairy cow milk by combining grazing with feeding of total mixed ration. *J.Dairy Res.,* 77, 225-230. 10.1017/S002202991000004X.

13. O'CALLAGHAN, T.F., HENNESSY, D., MCAULIFFE, S., KILCAWLEY, K.N., O'DONOVAN, M., DILLON, P., ROSS, R.P., STANTON, C., 2016, Effect of pasture versus indoor feeding systems on raw milk composition and quality over an entire lactation. *J. Dairy Sci.* 99, 9424-9440. 10.3168/jds.2016-10985.

14. PLATA-PÉREZ, G., ANGELES-HERNANDEZ, J.C., MORALES-ALMARÁZ, E., DEL RAZO-RODRÍGUEZ, O.E., LÓPEZ-GONZÁLEZ, F., PELÁEZ-ACERO, A., CAMPOS-MONTIEL, R.G., VARGAS-BELLO-PÉREZ, E., VIEYRA-ALBERTO, R., 2022, Oilseed supplementation improves milk composition and fatty acid profile of cow milk: a meta-analysis and meta-regression. *Animals* 12, 1642. doi.org/10.3390/ani12131642.

15. RADONJIC, D., DJORDJEVIC, N., MARKOVIC, B., MARKOVIC, M., STESEVIC, D., DAJIC-STEVANOVIC, Z., 2019, Effect of phenological phase of dry grazing pasture on fatty acid composition of cows' milk. *Chil. J. Agric. Res*. 79(2), 278-287. 10.4067/S0718-58392019000200278.

16. REGO, O.A., CABRITA, A.R.J., ROSA, H.J.D., ALVES, S.P., DUARTE, V., FONSECA, A.J.M., VOUZELA, C.F.M., PIRES, F., BESSA, R.J.B., 2016, Changes in milk production and milk fatty acid composition of cows switched from pasture to a total mixed ration diet and back to pasture. *Ital. J. Anim. Sci*., 15(1), 76-86. 10.1080/1828051X.2016.1141330.

17. SANTA, A., MIERLITA, D., DARABAN, S., SOCOL, C.T., VICAS, S.I., SUTEU, M., MAERESCU, C.M., STANCIU, A.S., POP, I.M., 2022, The effect of sustainable feeding systems, combining total mixed rations and pasture, on milk fatty acid composition and antioxidant capacity in jersey dairy cows. *Animals,* 12, 908. doi.org/10.3390/ani12070908.