European Journal of Agricultural and Rural Education (EJARE)
Available Online at: https://www.scholarzest.com
Vol. 5 No. 02, February 2024
ISSN: 2660-5643

STUDY OF THE RELATIONSHIP BETWEEN THE PERCENTAGE OF FATTY ACIDS IN MILK´S FAT AND BLOOD PLASMA DURING THE STAGES OF MILK PRODUCTION IN HOLSTEIN-FRIESIAN COWS

Zainab S. Al-Obaidi
A. M. Dr. Muntaha Y. Yousief
Department of Animal Production, College of Agriculture, University of Basrah, Iraq
email: zaineb@utq.edu.iq
email: muntaha.yousief@uobasrah.edu.iq

Article history:

Received: 14th December 2023
Accepted: 10th January 2024
Published: 18th February 2024

Abstract:

The relationships between fatty acids in milk fat and the quality standards of milk production during the lactation phase are very weak in dairy cows. Therefore, the current study aimed to predict the correlation between the studied standards and the proportions of milk components through the contribution ratios of fatty acids and biochemical blood standards during different production stages. This study was conducted at the Taj Al Nahreen Cattle Station in Al-Qadisiyah Governorate. The cows were divided into three groups, from which 720 samples of cow's milk were collected for a period of 90 days from Holstein-Friesian cows. Additionally, 360 blood samples were collected from 60 Holstein-Friesian cows to determine the biochemical traits. The fatty acid components were studied for the entire study period, and the results showed significant differences in the proportions of saturated and unsaturated fatty acids in blood plasma during the production stages. The proportion of saturated fatty acids in blood plasma increased compared to unsaturated fatty acids, depending on the production stages

Keywords: fatty acids, milk production, blood plasma

INTRODUCTION:

It has been established that the presence of essential fatty acids plays a beneficial role in a wide range of diseases in humans and animals, including dairy cows (Moallem, 2018; Muntaha et al., 2023). Moreover, the fatty acids present in milk fat have economic importance. Recent advancements in analysis methods and increased efficiency have enabled researchers to study milk components not only for scientific purposes but also for practical technological applications (Chung et al., 2018; Karrar et al., 2023) , The fatty acids found in milk fat are considered important dietary components for a large portion of the population. In the past, it was thought that they could have a negative impact on human health, but over the past decade, this concept has been significantly reassessed. Currently, the impact of milk fat on human health is seen as more positive than it was in previous periods (Chung et al., 2018; Raed et al., 2023).

MATERIALS AND METHODS:

The milk samples were collected from the milk of cows during the experimental period from Holstein Friesian cows belonging to Taj Al-Nahrain Company in Al-Qadisiyah Governorate. The samples were collected from the morning milking once a week for each cow (60 ml) at 7:30 am in sample collection bottles and stored in the freezer at a temperature of (-18) °C. 360 blood samples were collected from all cows, including 180 samples from 60 Holstein-Friesian cows to analyze the biochemical characteristics, and 180 blood samples were placed in specialized blood collection tubes containing EDTA anticoagulant to study the fatty acid components in them for all months of the experiment. Blood components and milk components were measured, and then the following steps were performed:

First: Estimation of the fatty acid percentages in cow's milk using gas chromatography. The samples were prepared and esterified to extract the milk fat and analyze the fatty acids present in it. The milk fat was separated by adding 10 ml of separation funnel, along with adding 10 ml of 95% ethanol and then adding 25 ml of diethyl ether. Then, 25 ml of petroleum ether was added, and the mixture was shaken well until it separated into two layers. After that, only the alcohol layer which contains the fat was taken, and the alcohol was evaporated completely. Then, the sample was prepared by esterifying triglycerides using their reaction with methyl potassium hydroxide solution. The total fatty acids of cow's milk fat were analyzed in the Clinical Chemistry Laboratory, Faculty of Pharmacy, University of Basra, using a gas chromatography device (SHIMADZU, Japan) equipped by the GC Solutions program, and the injection was performed using an electric injector. After obtaining the spectrum of each compound, the results were processed using the GC Solutions program.

Second: Estimation of fatty acids in the blood. Blood samples were taken from the cows in the morning after milking. 10 ml of blood was taken from the jugular vein of each cow into a dry, clean tube without anticoagulants, and then it was cooled down. They were centrifuged at 3000 × g for 20 minutes. The serum was separated into 1.5 ml of clean and dried Eppendorf tubes (Eppendorf AG, Hamburg, Germany), and they were frozen at -20 degrees Celsius until the time of analysis. The free fatty acids in the blood serum were determined using chromatographic study using the Chromatec-Crystal 5000 Gas Chromatograph (Chromatec, Yoshkar-Ola, Russian Federation), equipped with a flame ionization detector. The length of the quartz capillary column (Restek, Bellefonte, PA, USA) was 60 meters, with an inner diameter of 0.25 mm. The stationary phase was FFAP, and the film thickness was 0.25 micrometers. The relationships between the studied variables were calculated using simple and stepwise multiple regression within the SPSS statistical program (2019)

$y= a+bx$

$y= a+b1x1 +b2x2 ....+bnxn$

The correlations were calculated using the Orgien Pro program (2021) between blood parameters, milk production, and its components.

RESULTS AND DISCUSSION

Percentages of fatty acids in milk for different stages of milk production:

The saturated fatty acids were dominant over the unsaturated fatty acids regardless of the production stage , The percentages of fatty acids in milk were significantly associated ($P<0.05$) with the stages of milk secretion. The mid-production stage showed a significant superiority ($P<0.05$) in the percentages of saturated fatty acids, including Lauric acid (C12:0), Myristic acid (C14:0), Pentadecanoic acid (C15:0), Palmitic acid (C16:0), and Stearic acid (C18:0), which constitute high proportions of saturated fatty acids , In general, Holstein-Friesian cows exhibited a notable increase in total unsaturated fatty acids. This is because this breed has been carefully selected and intensively improved to increase milk production, which led to a decrease in fat percentage due to a negative correlation between them. Consequently, improving milk production also enhances the quality of milk by increasing the proportions of various unsaturated fatty acids.

Table (1) Average percentages of fatty acids in milk for different stages of production ± standard deviation

Acids	Production start period	Mid-Production period	End of production period
l C12:0 (Lauric acid	1.795±0.1c	4.375±0.2a	3.581±0.2b
C14:0 (Myristic acid)	10.388±0.4c	14.683±0.2a	11.171±0.4b
C15:0 (Pentadecanoic acid)	1.674±0.07c	2.638±0.1a	2.293±0.1b
C16:0 (palmitic)	32.164±0.09c	33.723±0.09a	33.062±0.10b
C17:0 (Margaric acid)	0.705±0.14a	0.592±0.15b	0.485±0.11c
C18:0 (stearic)	7.734±0.08c	9.034±0.06a	8.481±0.09b
C14:1 c9(myristoleic)	1.363±0.07c	2.851±0.27a	2.237±0.06b
) lC16:1 c9 (palmitoleic	1.153±0.2c	1.280±0.33a	1.210±0.1b
lC18:1 c9 (oleic)	18.131±0.2a	17.836±0.2c	17.990±0.1b
18:2 c9,c12(linoleic)	2.703±0.15a	2.349±0.2c	2.557±0.08b
18:2 c9,t11 (Rumenic acid)	0.613±0.06	0.647±0.05	0.681±0.04
18:3 c9,c12,c15 (linolenic)	0.815±0.06a	0.642±0.17b	0.488±0.05c
20:3 c8,c11,c14 (Eicosatrienoic acid)	0.174±0.13a	0.103±0.2b	0.053±0.09c

22:4 c7,c10,c13,c16 (arachadonic)	0.207±0.01a	0.118±0.03b	0.072±0.02c
SFA	54.460±0.06c	65.045±0.12a	59.073±0.05b
MUSFA	20.646±0.3c	21.967±0.4a	21.436±0.16b
PUSFA	4.512±0.33a	3.859±0.51b	3.817±0.15b
USFA	25.158±0.06b	25.826±0.12a	25.253±0.05b

Percentages of fatty acids in blood plasma for different stages of production:

The fatty acid ratios in blood plasma for different stages of production are shown in Table 2. There were significant differences in the saturated and unsaturated fatty acid ratios in blood plasma for the different stages of production (beginning, middle, and end of production). The proportion of saturated fatty acids in blood plasma was higher than unsaturated fatty acids, with significant associations ($P<0.05$) with different stages of milk production. The end of production stage showed significant superiority ($P<0.05$) in the proportion of saturated fatty acids for butyric acid (c12:0), pentadecanoic acid (C15:0), and palmitic acid (C16:0), which constitute a high percentage of saturated fatty acids (0.721, 11.076, 18.893, respectively). This was compared to the beginning of the production stage, which had percentages of 0.596, 10.669, and 18.113, respectively, in blood plasma of Holstein cows. On the other hand, there were no significant differences in the proportions of margaric acid (C17:0) and stearic acid (C18:0) across the different stages of production (beginning, middle, and end). This did, however, have an effect on the overall proportion of saturated fatty acids (SFA), which was significantly higher at the end of production (48.245) compared to the middle (47.611) and beginning (47.001) stages. As for the proportions of monounsaturated fatty acids (MUSFA) mentioned in Table 10, the beginning of the production stage showed significant superiority ($P<0.05$) in the proportions of myristoleic acid (C14:1c9), oleic acid (C18:1c9), linoleic acid (C18:2c9c12), conjugated linoleic acid (C18:2c9c11), linolenic acid (C18:3c9,c12,c15), eicosatrienoic acid (C20:3c8,c11,c14), and arachidonic acid (C22:4c7,c10,c13,c16), which were 0.969, 12.306, 21.2366, 0.259, 5.903, 0.526, 1.602%, respectively, in their production during the middle stages (0.947, 12.17, 21.046, 0.178, 5.819, 0.459, 1.502%) and the end of production (0.927, 12.061, 20.870, 0.081, 5.735, 0.414, 1.400%). Palmitoleic acid (C16:1c9) showed significant superiority ($P<0.05$) in the middle of production, with a proportion of 1.717%, compared to the beginning (1.646%) and end (1.751%) stages. The same table showed the superiority of monounsaturated and polyunsaturated fatty acids in the beginning of the production stage, with proportions of 14.920 and 29.524%, respectively, compared to the middle stages (14.835 and 29.003%) and the end of production (14.739 and 28.500%). The changes in the patterns of fatty acids in blood plasma are due to changes that occurred in milk fat. The concentration of fatty acids in plasma only affects long-chain fatty acids compared to essential fatty acids. This highlights the reason for the increase in the proportion of unsaturated fatty acids (monounsaturated and polyunsaturated) at the beginning of the production stages and their decrease as the stages progress. The proportions of fatty acids in the blood plasma of cows increase significantly after calving (Rossella et al., 2020), explaining the higher proportions of fatty acids in the blood plasma of cows at the beginning of the production stage. Afterward, there is a gradual decrease in the middle and end stages of production, as the liver starts to remove NEFA molecules resulting from fat breakdown and their reduced formation. This leads to their utilization for energy production, ketone body formation, or conversion into glycerol-3-phosphate.

Table (2) Average percentages of fatty acids in blood plasma for different stages of production ± standard deviation

Acids	Production start period	Mid-Production period	End of production period
C12:0 (Lauric acid	0.569±0.15c	0.649±0.2b	0.721±0.07a
C14:0 (Myristic acid)	1.239±0.05a	1.156±0.2b	1.066±0.18c
C15:0 (Pentadecanoic acid)	10.669±0.05c	10.857±0.06b	11.067±0.11a
C16:0(palmitic)	18.113±0.13c	18.432±0.16b	18.893±0.14a
C17:0 (Margaric acid)	0.672±0.02	0.679±0.03	0.668±0.04

C18:0 (stearic)	15.841±0.58	15.840±0.10	15.832±0.05
C14:1 c9(myristoleic)	0.969±0.10a	0.947±0.08b	0.927±0.10c
C16:1 c9 (palmitoleic)	1.646±0.35b	1.717±0.10a	1.751±0.45a
C18:1 c9 (oleic)	12.306±0.13a	12.171±0.26b	12.061±0.11c
18:2 c9,c12(linoleic)	21.236±0.02a	21.046±0.01b	20.870±0.02c
18:2 c9,t11 (Rumenic acid)	0.259±0.10a	0.178±0.10b	0.081±0.16c
18:3 c9,c12,c15 (linolenic)	5.903±0.14a	5.819±0.10b	5.735±0.10c
20:3 c8,c11,c14 (Eicosatrienoic acid)	0.526±0.05a	0.459±0.12b	0.414±0.10c
22:4 c7,c10,c13,c16 (arachadonic)	1.602±0.07a	1.502±0.12b	1.400±0.11c
SFA	47.001±0.02c	47.611±0.01b	48.245±0.01a
MUSFA	14.920±0.10a	14.835±0.1	14.739±0.16
PUSFA	29.524±0.07a	29.003±0.08b	28.500±0.12c
USFA	44.444±0.07b	43.838±0.12a	43.239±0.11c

Correlation coefficients between milk production, its components, and fatty acids in milk:

Figure (1) shows that the correlation coefficients are very low and did not reach significance between protein, non-fat solids, saturated and unsaturated fatty acids in milk. However, a positive and highly significant correlation ($P<0.001$) was found ranging from 0.81 to 0.90 between monounsaturated and polyunsaturated fatty acids and overall fatty acid content in milk.According to Kirchnerová (2012), Foltys and Kirchnerová (2012), other significant correlation coefficients were found: $r=0.307$ for milk, $r=0.353$ for fat, and $r=0.340$ for proteins, primarily resulting from myristic acid (C14:1), which was the closest estimated acid to the production level during the previous lactation period. The correlation coefficients were $r=0.597$ for days, $r=0.481$ for milk, $r=0.431$ for fat, and $r=0.508$ for proteins , Furthermore, there was a negative and significant correlation ($P<0.001$) between saturated fatty acids and fat content in milk, with a value of 0.89 in this direction. Short-chain fatty acids up to C10 are derived from the biosynthetic process in the mammary gland. Myristic acid is considered the building block that arises during fermentation processes in the rumen. In the reductive condensation process, a long chain is formed in the form of acetyl CoA, resulting in higher and higher fatty acid formation with an even number of carbon atoms (Jenkins & Mcguire, 2006). A significant decrease in polyunsaturated fatty acid content ($r=-0.418$) was found with an increase in daily fat production. There were also statistically significant relationships between the total sum of polyunsaturated fatty acids and fat content in milk ($r = 0.321$; $P < 0.05$) and lactose content in milk ($r = 0.458$; $P < 0.01$). However, it is important to evaluate n6 and n3 fatty acids separately within this group. It is also expected that fat content will increase. The analysis of the relationships between milk fat fatty acids and milk quality production criteria shows very weak correlations between fatty acids and lactation stage and milk quality production criteria in dairy cows."

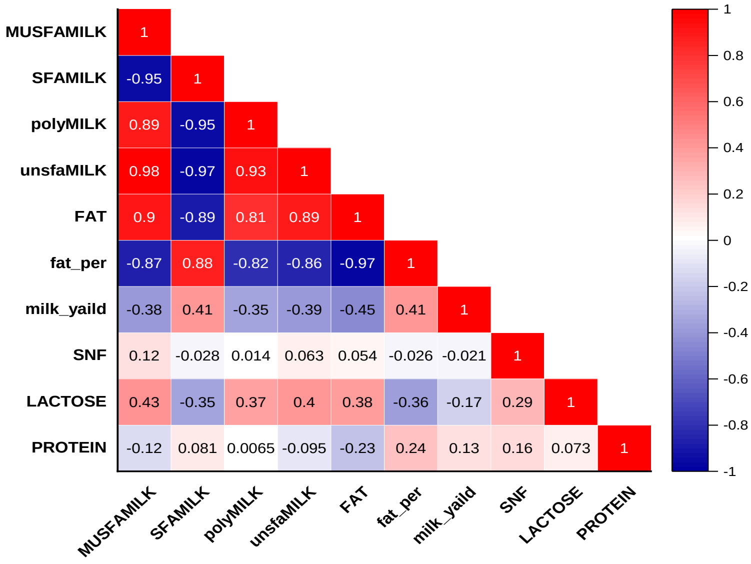

Figure (1) Correlation coefficients between milk production, its components, and fatty acids in milk

Correlation coefficients between the amount of milk fat and fatty acids in milk and blood plasma:

The correlation coefficients between milk fat content, milk fatty acids, and plasma fatty acids are shown in Figure (2). A highly significant positive correlation (P>0.001) is observed between milk fat content and saturated and unsaturated fatty acids in plasma, with coefficients of 0.71 and 0.84, respectively. Additionally, there is a highly significant positive correlation (P>0.01) between milk fat content and polyunsaturated fatty acids in plasma, as well as saturated fatty acids in milk. On the other hand, a highly significant negative correlation was found between milk fat content and monounsaturated fatty acid ratio in plasma and milk, as well as polyunsaturated fatty acids and overall fatty acids in milk, with coefficients ranging from 0.71 to 0.86

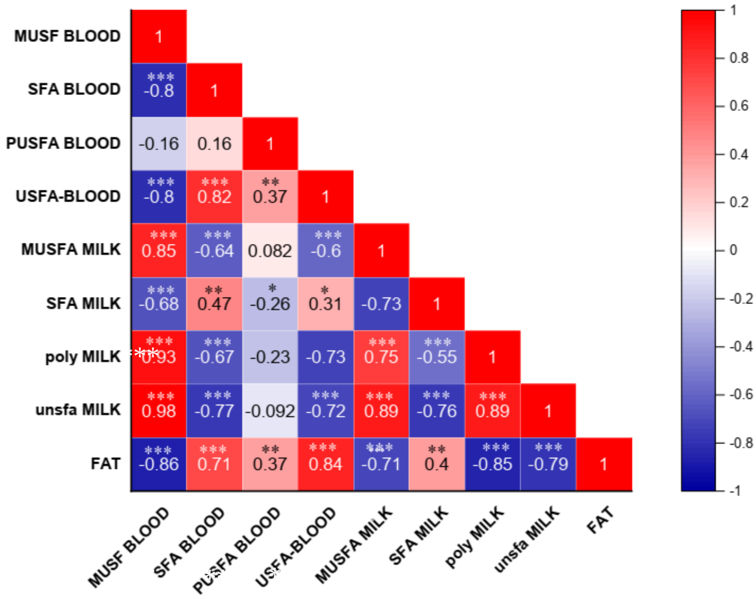

Figure (2) Correlation coefficients between the amount of milk fat and fatty acids in milk and blood plasma

The relationship between saturated and unsaturated fatty acids in milk and blood plasma at different stages of production:

The relationship between saturated and unsaturated fatty acids in milk and plasma at different stages of production is discussed in the text. Figure 3 illustrates a non-linear (quadratic) and statistically significant ($P<0.05$) relationship between the percentage of saturated fatty acids in milk and the milk production stage. The milk production stage contributes to 99.72% of the variation in the percentage of saturated fatty acids in milk. The proportion of unsaturated fatty acids in milk increased from the beginning of the production stage and reached its highest point at the middle of the production stage, with an average of 35.42%. Then, the percentage of saturated fatty acids started to decrease at a rate of 8.2785% in milk from the middle of the milk production stage to its end. Figure () shows a linear relationship between the milk production stage and the percentage of unsaturated fatty acids in plasma. It is evident from the figure that there is a positive relationship between the percentage of unsaturated fatty acids in plasma and the advancement of the production stage, with an average increase of 0.622%. The precision degree (contribution of the milk production stage to the variation in the percentage of unsaturated fatty acids in plasma) was 99.99%. Figure 4 illustrates the relationship between unsaturated fatty acids in milk and plasma at different stages of milk production. The relationship was non-linear (quadratic) and statistically significant ($P<0.05$), with the milk production stage contributing to 97.04% of the variation in the percentage of unsaturated fatty acids in milk. The proportion of unsaturated fatty acids in milk increased from the beginning of the production stage and reached its highest point at the middle of the production stage, with an average of 28.226%. Then, the percentage of unsaturated fatty acids started to decrease at a rate of 8.738% in milk from the middle of the milk production stage to its end. There was a linear relationship between unsaturated fatty acids in plasma and the milk production stage. It is evident from the figure that there is a positive relationship between the percentage of unsaturated fatty acids in plasma and the advancement of the production stage, with an average increase of 0.6025%. The precision degree (contribution of the milk production stage to the variation in the percentage of unsaturated fatty acids in plasma) was 100%.

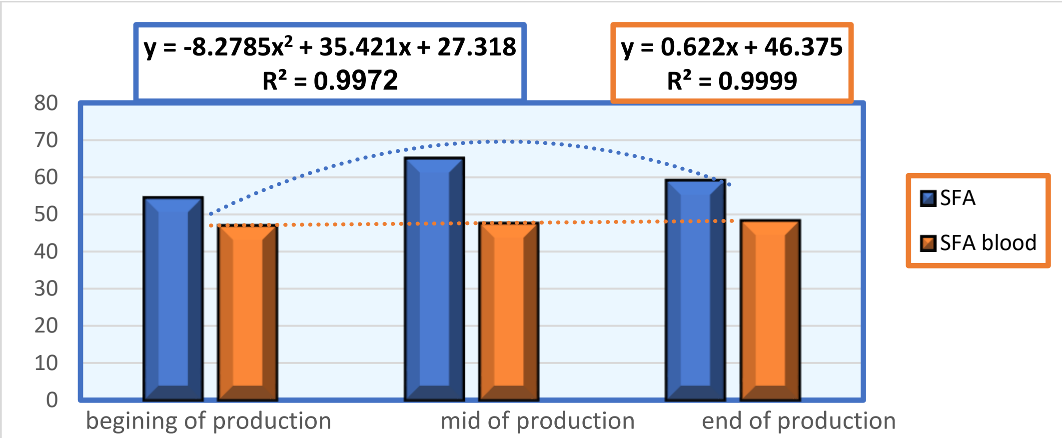

Figure (3) The non-linear (quadratic) relationship between the fatty acids in milk and the linear relationship between the fatty acids in the blood plasma

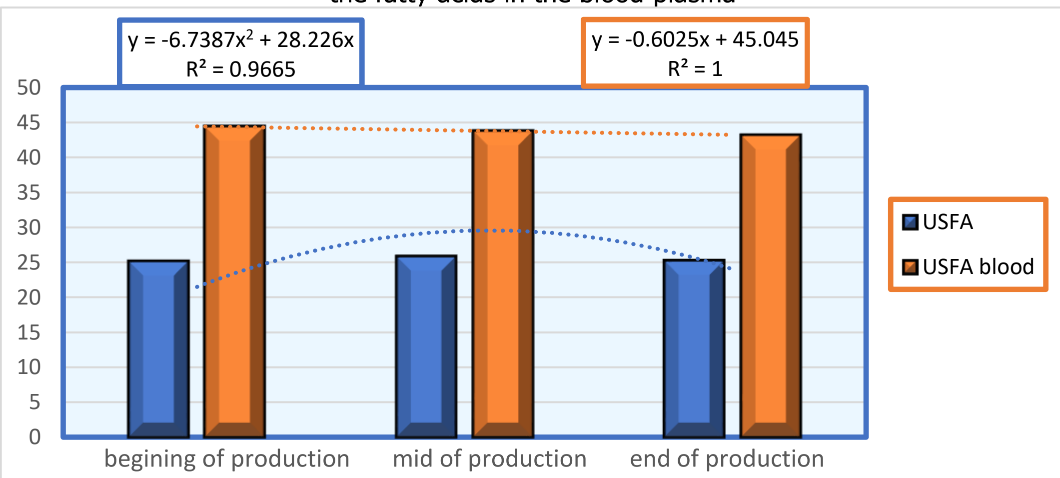

Figure (4) The non-linear (quadratic) relationship between trans fatty acids in milk and the linear relationship between trans fatty acids in blood plasma

CONCLUSIONS:

Based on the information provided, we can conclude that there is a positive correlation between the amount of fat and the concentration of polyunsaturated fatty acids in plasma, as well as the concentration of saturated fatty acids in milk. Additionally, there is a negative correlation between the amount of fat in milk and the percentage of monounsaturated fatty acids in plasma and milk, as well as the total unsaturated fatty acids in milk.

REFERENCES :

1. **Chung, I.-M.; Kim, J.-K.; Lee, K.-J.; Son, N.-Y.; An, M.-J.; Lee, J.-H.; An, Y.-J.; Kim, S.-H. 2018 .** Discrimination of organic milk by stable isotope ratio, vitamin E, and fatty acid profiling combined with multivariate analysis: A case study of monthly and seasonal variation in Korea. Food Chem., 261, 112–123.

2. **Foltys V. and K. Kirchnerová , 2012** . IMPACT OF LACTATION STAGE AND MILK PRODUCTION ON MILK FAT FATTY ACIDS RATIO , Slovak J. Anim. Sci., 45, 2012 (1): 30-35 © 2012 CVŽV ISSN 1337-9984
3. **Hanus , 0. ; Samkova, E. ; Spicka , J ; Sojkova, K. ; Hanusova , K. ; Kopec , T ; Vyletelova , M. jedelska , R. 2010.** Relationship between concentration of health important groups of fatty acids and components and technological properties in cow milk. Acta Univ. Agric. et Silvic. Mendel. Brun., vol. LVIII, 2010, no. 5, p. 137-154.
4. **Haubold, S., C. Kröger-Koch, A. Starke, A. Tuchscherer, A. Tröscher, H. Kienberger, M. Rychlik, U. Bernabucci, E. Trevisi, and H. M. Hammon. 2020**. Effects of abomasal infusion of essential fatty acids and conjugated linoleic acid on performance and fatty acid, antioxidative, and inflammatory status in dairy cows. J. Dairy Sci. 103:972–991.
5. **Haubold, S., C. Kröger-Koch, A. Starke, A. Tuchscherer, A. Tröscher, H. Kienberger, M. Rychlik, U. Bernabucci, E. Trevisi, and H. M. Hammon. 2020**. Effects of abomasal infusion of essential fatty acids and conjugated linoleic acid on performance and fatty acid, antioxidative, and inflammatory status in dairy cows. J. Dairy Sci. 103:972–991
6. **Jenkins , T .C. and McGuire , M .A .2006**. Major advances in nutrition: impact on milk composition. J. Dairy Sci., 89: 1302–1310.
7. **Karrar T. Dragh ; Raghdan H. Mohsin ; Muntaha Y. Yousief , 2023** . Relationship of genotypes of csn3 gene in milk production and proportions of its components in Holstein Friesian cows bred in Iraq , Journal of Survey in Fisheries Sciences 10(3S) 3740-3745
8. **Moallem, U. 2018.** Invited review: Roles of dietary n-3 fatty acids in performance, milk fat composition, and reproductive and immune systems in dairy cattle. J. Dairy Sci. 101:8641–866
9. **Moallem, U.; Lehrer, H.; Livshits, L.; Zachut, M. 2020** . The effects of omega-3 α-linolenic acid from flaxseed oil supplemented to high-yielding dairy cows on production, health, and fertility. Livest. Sci. 2020, 242, 104302
10. **Muntaha Y. Youssef ; Hanaa A. Al-Ghalibi and Haider A. Al-Battat , 2023** . Relationship between genetic polymorphisms of the DGAT1 gene and fatty acid production in the milk of cows raised in Iraq (local, local, and Holstein Friesian) , Iraqi Journal of Veterinary Sciences , 1607-3894 .
11. **Raed K. Al-Muhja ; Muntaha Y. Yousief ; Allawi L. Dagher Al-Khauzai .2023 .** A Study of the Relationship of Genetic Polymorphisms of the PRL Gene with Milk Production and its Components in Holstein Friesian Cows , Texas Journal of Agriculture and Biological Sciences ISSN NO: 2771-8840.
12. **Rossella Tessari, Michele Berlanda , Massimo Morgante, Tamara Badon, Matteo Gianesella, Elisa Mazzotta, Barbara Contiero and Enrico Fiore , 2020**. Changes of Plasma Fatty Acids in Four Lipid Classes to Understand Energy Metabolism at Different Levels of Non-Esterified Fatty Acid (NEFA) in Dairy Cows , Animals 2020, 10, 1410; doi:10.3390/ani10081410 .
13. **Toni, F.; Vincenti, L.; Grigoletto, L.; Ricci, A.; Schukken, Y.H. Early lactation ratio of fat and protein percentage in milk as associated with health, milk production, and survival. J. Dairy Sci. 2011**, 94, 1772–1783. [CrossRef] 11. Bastin, C.; Gengler, N.; Soyeurt, H. Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. J. Dairy Sci. 2011, 94, 4152–4163.