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Research Article

Ryegrass Hay Versus Whole Crop Silage: Effects On Milk C18:3 n-3 in Malta

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Abstract

Cattle are fed a variety of diets around the globe, primarily depending on the producer and the location. Local whole crop silage and hay are the two major types of feed that farmers in Malta are accustomed to using. Ryegrass, an imported crop that is growing in popularity, is now being used by certain farmers. The goal of this study is to identify the benefits and drawbacks of each type of feed within a certain setting. For this investigation, two farms from which milk samples were obtained were taken into consideration. Farm A cows were fed on whole crop while Farm B relied on ryegrass. Although this was not statistically significant, quantitative analysis revealed that Farm B produced milk with a superior fatty acid profile. Such a study undoubtedly offers new avenues for farmers' perspectives as well as for producers working with the herding of cattle. This study may be considered to be a pilot study for a more comprehensive investigation into the quality of locally produced fresh fodder and imported fodder that may contribute to the improvement of milk on local farms.

Introduction

Dairy cattle farms are distributed all around the islands of Malta and Gozo, which are two main inhabited islands of the Maltese archipelago at the centre of the Mediterranean Sea. According to the National Statistics Office [1], in 2020 there were approximately 241 cattle farms in Malta and Gozo, with 14,447 heads, with 5,995 dairy cows. Some producers also rear sheep and goats on the same farm. The most commonly bred dairy cattle breeds include the Holstein-Friesian, the Jersey, the Brown Swiss and the Estonian Red Cattle breeds [2]. The rumen is an ample-sized fermentation chamber harbouring bacteria, protozoa and fungi. These organisms form a symbiotic relationship to digest plant fibres, playing a role in the deposition of Polyunsaturated Fatty Acids (PUFAs) into animal products. When plants lipids are exposed to the rumen microbes, they undergo hydrolysis and biohydrogenation [3]. The main role of these processes is to change Unsaturated Fatty Acids (UFA) such as linolenic acid (C18:3 n-3) and linoleic acid (C18:2 n-6) into saturated fatty acids (SFA; C18:0). N-3 and N-6 are incorporated into meat and milk of ruminants before the saturation process has been completed [4]. Different types of feeding strategies are used in Malta. In fact, these are mainly based on imported forage such as hay, alfalfa, ryegrass and local whole crop which includes baleage or silage, as it is commonly referred to locally. Approximately half of the daily feeding ration comprises of between 10 to 14 kilograms of concentrates.

Silage is made from different types of plants and in different seasons [5], so that the farmer always has fresh silage all year round. On the other hand, wheat, ryegrass and clover are seeded in October and are usually harvested for silage at around March, depending on the weather. The wheat and ryegrass are harvested when early blooms appear, this is the stage of maturity, whereas clover is harvested when the first flower dries up. In the case of corn, it is seeded at around May or June, again always depending on the weather. By this time, it will be summer and in Malta, this is very dry, so the farmers have to irrigate the corn once every two days until the corn cob is formed. Wheat and ryegrass are harvested and stored for about two days before use to dry and achieve a higher dry matter content. On the other hand, after harvest, clover has to be left for four days as it has a thicker stem than wheat and ryegrass. As for the corn the farmer stops irrigating it from one week prior to harvesting, then stored for four days until it wilts. Baling is performed by a machine, where farmers pass over the grass with the baler. The baler grabs grass and cuts it into 10-cm lengths. These are then compressed into a round bale. Once the bale is made, it must be wrapped into a special type of plastic in the shortest time possible. For the process of silage, a 'bale wrapper' is used. This machine covers the bale with plastic. In fact, this makes forty two rounds of plastic so that no air can enter. In spite of this, correct storage is required so as to prevent spoilage. Some researchers argue that the production of silage is not always viable, and one should consider economic and environmental factors. The quality of the silage as feed may impact on the nutritional content and the quality of milk [6]. This system is becoming increasingly popular on the islands. The use of hydroponic grass as an alternative to the orthodox ration [7] is another system which is also practised by only some farmers. When silage is not practised, farmers obtain forages, like ryegrass, through importation. Some farmers resort to the imported source, as they believe that silage is laborious and, in some situations, risky due to potential post-harvest spoilage [8]. Whereas silage production is time consuming, the importation of ryegrass is a readily available and a secure source though more expensive. Additionally, the limitations of land availability for the growing of fodder crops is another setback for local farmers and so forage importation is the only option. Currently the current cost of feed ration is approximately € 7.00 per cow daily which is relatively high when compared to the cost of ration in other European Union member states.

The quality of bovine milk on the Maltese Islands has been previously studied in comparison with ovine and caprine milk [2,9]. In one of the studies related to the mineral content of milk, bovine milk was superior in calcium, zinc and barium as compared to the other two types [2]. In terms of proximate analysis, the characteristics of bovine milk were relatively similar to those of caprine milk. For most of the proximate parameters, ovine milk was superior to the bovine and caprine milk types [9]. As with other EU consumers, the local consumer prefers the consumption of local bovine milk [10]. Besides the long tradition of local bovine milk production, local milk reaches the consumer within twenty-four hours from the milking of the cows. Due to the potential contamination of milk by *Brucella melitensis* (discovered in Malta by Sir David Bruce and Sir Themistocles Zammit) [11], the milk is pasteurised at 71 °C for 15 seconds. The local consumer distinguishes this fresh milk from the Ultra-High Temperature (UHT) treated milk imported from abroad. (UHT) treatment has been known to alter the nature and the nutritional value of the milk [12]. In spite of this, this study focuses on the nutritional value of local milk, in terms of fatty acid profile, in relation to feed intake; forage ryegrass and local whole crop silage. In particular, this study assesses and identifies the C18:3 proportion of milk produced by cows consuming two different forages. The aim of this study is based on the hypothesis that if feeding ryegrass proves to actively yield milk rich in N-3, then there is potential to promote the use of high-quality forage in cows' ration and hence the possibility of marketing local n-3 milk.

Materials and Methods

In this study two different farms were investigated. Farm A is referred to as the farm feeding a silage ration to the herd while Farm B is the farm feeding a ryegrass ration to its herd. Moreover, Farm A also includes two kilograms of fescue per cow in the ration, which is not included for the ration for Farm B. Both farms feed local straw to their cows and they also feed the same type of concentrates but in different amounts (Table 1).

Table 1: Composition of the experimental diets (% of DM) of Farm A and Farm B.

	Farm A – Silage Diet	Farm B – Rye Grass Diet
Feed	%	%
Ryegrass	0	41
Local Straw	16	11
Normal	28	26
Special Dairy feed	14	22
Fescue	7	0
Silage	35	0
Total	100	100

Collection of milk samples

In all 14 samples from each farm were collected with the first sample from each farm was collected on the 27th of January. From the 27th of January till the 2nd of February, a milk sample from the bulk tank was collected daily from each farm to obtain seven samples from each farm. Until the seventh sample was collected the milk samples were stored in a -14 °C freezer. All milk samples were sent to the Associazione Regionale Allevatori della Lombardia, an accredited lab in Italy to obtain the fatty acid profile in the milk samples. The extraction of the lipids and lipid-soluble compounds was performed in conformity with ISO 14156:2001/AMD 1:2007, the preparation of fatty acid methyl esters from milk fat in accordance with ISO 15884:2002 and the determination of the fatty acid composition by gas-liquid chromatography in conformity with ISO 15885:2002. The same procedure was followed after a month to obtain another milk fatty acid profile.

Collection of feed samples

Four different feed samples were collected, i.e. ryegrass, silage, fescue and local straw. Good homogeneity in the sampling was ensured by having various samples from various parts of the bales. The samples were analysed for proximate parameters using a NIR spectrophotometer – SpectraStar 2400 (Unity Scientific). The samples were transferred to a quartz cup and the proximate values were obtained after a three-run cycle with thirty readings per run.

Statistical analysis

Statistical analysis was conducted in order to determine any relevant difference between the two farms for the quality of milk, in term of fatty acid quality and quantity. One-Way and Two-Way Anova were used (Prism, GraphPad V.5) for this determination. P values less than 0.05 were considered as significant.

Results and Discussion

Various research studies established that the C18:3 n-3 PUFA content in milk fat is increased under grass/pasture milk production systems when compared with conventional TMR based systems [13,14]. Ruminant products are relatively high in SFA despite the fact that ruminant forage-based diets are rich in PUFA [15-17]. This is mainly triggered by the microbial biohydrogenation of dietary UFA in the rumen. Thus, the main objective of this study was to investigate whether the ensiled local cereal crop would constitute a similar effect as that reported in other studies by suppressing biohydrogenation and produce milk fat with a richer C18:3 n-3 than ryegrass hay. Table 2 illustrates the proximate analysis and energy value for the silage and ryegrass diets. Table 3 represents the nutritional value of both rations. As an ingredient, was superior to local hay, fescue and wholecrop silage, in terms of dry matter, ash, fat content, but inferior in terms of neutral acid detergent fibre contents. However, it can be noted that NDF for silage of Farm A is higher than Farm B. However, the general NDF values in the ration are relatively similar. The higher the starch in the diet of the cows the more propionate there will be in the rumen [18]. If the propionate exceeds the butyrate/acetate levels, the volatile fatty acid ratio is disrupted. The higher the propionate levels in the rumen will hence knock out the fibrolytic bacteria that promote milk fat. Although the starch content of rations for both farms in this present study were within the range of other studies [19], the higher starch content for the silage-based ration (22.4%) than for the ryegrass-based ration (19.0%), the former seemed to have promoted higher amounts of propionates. However, this factor has not significantly contributed to the subsequent lower polyunsaturated fatty acids in milk derived from Farm A.

Table 2: Nutritional composition1 of local hay, ryegrass, fescue and silage.

	Silage Diet			Ryegrass Diet
	Local Hay	Fescue	Wholecrop Silage	Ryegrass
ME (MJ)	5.5	6	9	8.8
DM%	87.18	87.86	37.83	90.45
ASH%	8.4	10.88	9.89	10.15
CP%	7.65	9.82	8.22	9.21
EE%	2.26	2.54	2.17	2.61
NDF%	72.19	63.15	60.5	51.66
ADF%	36.98	31.19	39.04	23.3

[1]DM: Dry Matter; CP: Crude Protein; EE; Ether Extract; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber.

Table 3: Nutritive value of Farm A and Farm B rations.

	Farm A – Silage Ration	Farm B – Rye Grass Ration
ME(MJ)	208	217
Protein(%)	14.2	16.7
DUP(%)	5.4	5.6
Starch(%)	22.4	19
NDF(%)	37.5	34.7
NDF from forage (%)	28	27.2
Ash(%)	8.9	9.5

ME: Metabolizable Energy; DUP: Digestible Undegradable Protein; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber.

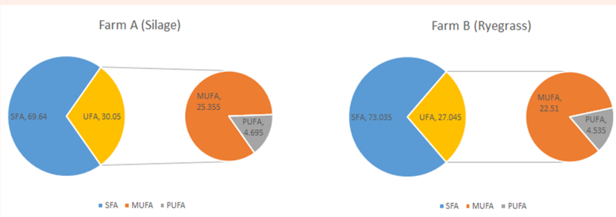
Volatile Fatty Acids (VFAs), acetic, propionic and butyric acids, are produced during ruminal fermentation, but are constantly removed by absorbed and transferred to the liver via the portal vein [20]. This mechanism is important in order to prevent the accumulation of VFAs that may result in the excessive lowering of the ruminal fluid pH. During the fermentation of ensiled cereals, VFAs are lost leading to aerobic instability. Clostridia will lower pH in silage, which will cause the deamination and breaking down of amino acids [21]. Although, the metabolic energy value of silage is higher than that of ryegrass (9MJ and 8.8MJ, respectively) (Table 2), the ration for farm B exhibited a higher energy value (217 MJ, vs 208MJ for farm A) (Table 3). The higher energy levels might have supplied the ruminal microbes. Indeed, volatile fatty acids are important products of fermentation in ruminant production since they contribute to about 72% of the total energy supply [22]. Table 4 presents the values of the major milk fatty acids of milk collected from the two farms during the trial. The values for the individual fatty acids were not statistically different between the two farms. The results yielded during this trial indicated that during the period January – March, cows consuming imported ryegrass hay (Farm B) as the main forage portion produced milk richer in UFA, particularly C18:3

n-3, whilst cows fed whole crop silage (Farm A) had higher SFA in milk, with lower C18:3 (Table 3). However, these results were not statistically different. There are various factors that might have affected the results of this study. These are mainly milk production and stage of lactation of the herd.

Table 4: Milk Fatty Acids for Farm A and Farm B.

		Farm A (Silage Ration)	Farm B (Ryegrass Ration)
butyric	C4:0	3.26±0.39	2.91±0.24
valeric	C5:0	0.03±0	0.035±0.005
caprionic	C6:0	2.265±0.305	2.205±0.155
	C8:0	1.425±0.195	1.485±0.085
	C10:0	3.135±0.385	3.555±0.125
	C12:0	3.505±0.325	4.075±0.025
myristic	C14:0	11.04±0.7	11.88±0.02
palmitic	C16:0	32.87±0.14	33.1±0.08
stearic	C18:0	9.34±0.4	8.335±0.015
oleic	C18:1 n-9	19.67±2.505	18.85±0.26
vaccenic	C18:1	1.05±0.55	1.555±0.025
linoleic	C18:2 n-6	3.285±0.015	2.735±0.085
linolenic	C18:3 n-3	0.285±0.005	0.425±0.015
	CLA	0.635±0.005	0.555±0.015

The 'Special Dairy' feed consists of rumen-protected fats supplied from calcium salts, which are designed to increase milk yield. Typically, they consist of a fatty acid profile of 44% C16 palmitic acid, 40% C18:1 oleic acid and 9.5% C18:2 linoleic acid. The higher amount of 'Special' concentrates consumed by the cows on Farm B might have in part contributed to the subsequent milk fat high in n-3 linolenic acid. Fat supplements in the diet, may contribute to higher fat content in milk. However, this is dependent on the fatty acid profile conveyed from the feed to the milk. The milk fat content may increase especially if there is the presence of C16 palmitic fatty acids, also known as the protected fatty acids [23]. Additionally, Farm B had higher milk C18:3 n-3 since the ration contained 22% of 'Special' concentrates while Farm A contained 14% of this rumen-protected fat rich concentrate. In a study [24], it was demonstrated that the supplementation of calcium salts altered the fatty acid concentration of milk fat in goats' milk, increasing levels of polyunsaturated and essential fatty acids. Figure 1 represents the fatty acid categories for both farms. There were no significant differences between the ration fatty acid categories for both farms, namely, saturated and the mono- and polyunsaturated fatty acids. The conservation of grass as silage may retain some of the benefits of fresh grass [25]. However, wilting is a crucial factor in the process of silage making. It is customary in Malta that the harvested crop is left to wilt for more than 48 hours, resulting in overexposure to sunlight with the consequential loss of the beneficial fats and vitamin E through oxidation. In fact, locally during silage making, no fermentable substrates are used. This may result in an inconsistent variation in the nutritive value of silage across time and from bale to bale.



%	SFA	UFA	MUFA	PUFA
Farm A	69.64±2.05	30.05±2.3	25.36±1.935	4.695±0.365
Farm B	73.04±0.855	27.05±1.475	22.51±1.61	4.535±0.135

Figure 1: Milk Fatty acid categories with farm.

However, this variance was not recorded for Farm B. In silage production and feeding process, losses of either dry matter or nutritive value can occur at the field level when cutting, mowing, wilting, chopping and baling. When ensiling or feeding out, aerobic spoilage occurs. In addition, annual ryegrass does not lose nutritive value as rapidly as most winter cereals [26]. The findings of this present go in accordance with those of Shingfield and co-workers [27] who reported that PUFA was significantly higher for hay than for silage diets. In this present study, although silage seemed to preserved more PUFA (4.695±0.3650% as compared to rye grass; 4.535±0.1350%), rye grass exhibited a higher content of α-linolenic acid (C18:3) than silage (0.425±0.015% and 0.285±0.005%, respectively). The extent to which these PUFA endure either ensiling or drying is unclear but there is some evidence that cows fed on either fresh grass or hay have higher levels of n-3 PUFA in their milk than cows fed on grass silage [28,29]. Although Shingfield and co-workers [30] reported that milk from cows consuming hay was richer in linoleic acid (C18:2) when compared to silage-based rations, in this present study the silage-based ration exhibited higher linoleic acid content in milk than that following ryegrass ration feeding. Comparing this inverse relation between the ryegrass and silage for linolenic and linoleic acids, indicates that linolenic acid has a higher transfer efficiency from diet to milk with ryegrass than with silage. A key factor that contributes to the biohydrogenation process are ruminal microbes. One of the transformations performed by the bacteria in the rumen is the microbial synthesis of the fatty acids [31,32]. These FA are meant to maintain a healthy fluidity of the microbial cell membrane [33]. The proportion of milk fat reflects rumen microbial activity and the respective proportions of different rumen microbial groups. Therefore, a shift or imbalance in the microbial population may affect the rumen proportions of VFA. Hence, the relation between VFA levels and the C18:3n-3 might have led to such results.

Initially, the ration of Farm B was richer in conjugated linoleic acids and α-linolenic acids. The potential to modify the milk fatty acid composition is by changing the diets [34]. Diary rations contain lipid supplements derived from plants that may provide a similar effect to those in the pasture. Plant lipid supplements may increase several trans-isomers of 18:1 and conjugated or non-conjugated 18:2, to silages and concentrate diets. Thus, in this current study, this might have contributed to higher polyunsaturated fatty acids in the silage ration rather than the rye grass ration (4.695±0.356 and 4.535±0.135 %, respectively). The difference in the quality and type of silage affects the subsequent milk PUFAs. Similarly, scientific literature claims that red clover silages produce a higher transfer efficiency of PUFA to the milk compared with other silages [35]. This is related to protein-bound phenols formed by polyphenol oxidase that may inhibit the activity of plant fats. A previous study [36] indicated that lower ruminal biohydrogenation of C18:3 n-3 to be associated with red clover clonal lines with high-PPO activity. Other studies claimed that the milk CLA differs between forages, with hay greater than grass silage [37]. However, in this study it was observed that silage contributed to higher conjugate linoleic acid than raygrass hay numerically. In fact, when considering the advantages of n-3s, it becomes clear that there is substantial interest in changing the fatty acid makeup of milk with the ultimate goal of enhancing the long-term health of customers [38].

Conclusion

Following the results obtained from this investigation, this appraisal suggests that cows consuming more than 40% of ryegrass produce milk rich in n-3. These promising results may present the local dairy processors with a novel concept of offering an initiative to the dairy farmers who include ryegrass to their dairy herd ration. Eventually, it has been observed that there is a potential of supplementing the diet of dairy cows with a significant portion of quality forage in order to improve milk quality in terms of n-3.

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