Article Type: Research
J Name: Modern Phytomorphology
Short name: MP
ISSN: ISSN 2226-3063/eISSN 2227-9555
Year: 2024
Volume: 18
Page numbers: 357 - 365
DOI: 10.5281/zenodo.200121
(10.5281/zenodo.2024-18-PDFNo.)
Short Title: Effect of milking frequency on the hygiene index and nutritional quality of raw milk: Mid-northern region Algerian study

RESEARCH ARTICLE

Effect of milking frequency on the hygiene index and nutritional quality of raw milk: Mid-northern region Algerian study

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Received: 05.12.2024, Manuscript No: mp-24-154343 | **Editor Assigned:** 13.12.2024, Pre-QC No. mp-24-154343(PQ) | **Reviewed:** 28.12.2024, QC No. mp-24-154343(Q) | **Revised:** 03.01.2025, Manuscript No. mp-24-154343(R) | **Accepted:** 09.01.2025 | **Published:** 15.01.2025

Abstract

The objective of this study was to determine the effects of reduced milking frequency on nutritional and microbiological cow's milk quality. Once-Daily Milking (ODM) on a standard diet; 3-Times Daily Milking on a standard diet (TDM) cows were assigned from 06 February to 30 May. Cows were on average 218 days into lactation at the start of the trial, and all cows were managed similarly throughout the trial. Milk yields and gross milk composition of cows on all treatments were measured. Results of this study showed a significant decrease in the average milk nutritional quality in TDM as compared to ODM as fat, lactose, protein, casein rates by 15%, 8%, 3.9% and 5.5% respectively. In addition, TDM decreased the minerals and vitamins levels by 5.8% as compared to milk obtained by ODM. In addition, the application of TDM in the milking affects the milk physical properties by the decrease of the milk conductivity, density and freezing point by 3.29%, 1.07% and 8.1% as compared to ODM milk. Moreover, the practice of TDM affects the milk bacteriological quality by the increase of Total Bacteria Count (TBC) (from 11.6 to 13.8), Total Aerobic Mesophilic Flora (TAMF) (from 6.4 to 7.8); Total Coliforms (TCol) (from 4.6 to 5.7); Fecal Coliforms (FCol) (from 3.2 to 4.2). Additionally, TDM increase significantly the Sulphite Reducing Clostridium (SRC), molds and yeasts count in milk as compared to ODM. In fact, the improvement of hygienic milking conditions through applying good hygiene practices and new techniques has made it possible to reduce the microbial load of the raw milk samples analysed bacterial flora. Therefore, the reduction in milk production in ODM was completely compensated by an increase in the concentration of milk components as fat, lactose, protein, casein, minerals and vitamins rates as compared to TDM.

Keywords: Bacterial flora, Improved conditions, Raw milk, Wholesomeness

Introduction

In dairy animals, daily milking is a prerequisite with reference to cattle health and management. The frequency of milking is considered to be an endpoint to determine the total milk yield. Around the globe, milking is done for a

frequency of once daily. Early lactation is meant to reduce metabolic stress whereas; late lactation alleviates the quality of farming life (Salama et al., 2003).

Milking cows three times a day means they are milked automatically following their natural daily patterns. Prior research has indicated that increased milking frequency leads to higher variable expenses, including labor, utilities, milking supplies, and extra feed costs. In Spain and other nations with extensive or semi-extensive goat production systems, high milking frequency poses a significant financial burden for dairy goat farms. In these contexts, milking less frequently enhances labor efficiency and minimizes the risks associated with milk storage.

Furthermore, we put forward this piece of work to come up with better strategies to get better yield of milk without any negative impacts improved conditions.

Thus, occasional milking must not negatively impact milk yield or quality over the long run in order to be a viable method. Furthermore, the method gives farmers more flexibility in their work schedules, which may lead to a better social life. However, it is unclear how this would affect the milk's nutritional and microbiological quality as well as the sustainability of production. In actuality, milk treatment can affect its quality, resulting in compositional and microbiological alterations, yield loss, and decreased dairy product quality. In addition to the improper use of milking equipment, low-quality milk is linked to the procedures used for milking, the health of the mammary glands, and herd management. On dairy farms, the availability of labor and the amount of time spent milking are important concerns. One potential method of lowering labor costs is to switch from Twice-Daily Milking (TDM) to Once-Daily Milking (ODM). Every aspect of society is watching the food business closely. According to Flores-Miyamoto et al. (2014), consumers are concerned about the safety, quality, and manufacturing process of products; raw milk drinking is now the primary way that germs and diseases are spread.

Since infections can compromise food safety and spoilage bacteria can shorten shelf life and impact the quality or output of milk products, microbiological contamination of milk is actually a significant problem (Flores-Miyamoto et al. 2014). Bacterial toxins produced by Bacillus, Clostridium, and Staphylococcus species are the other common causative agents in case of disease outbreaks (Zastempowska et al., 2016). Although the prevalence of foodborne pathogens in raw cow milk varies, numerous surveys have shown their presence, and human pathogenic verocytotoxin-producing Escherichia coli, Salmonella spp., and Campylobacter have all been linked to foodborne infections. 2% to 6% percent of bacterial foodborne outbreaks in developed nations is suggested to be caused by milk or milk products (Claeys et al., 2013).

In recent years, Algerian states witnessed alleviation in the quality of milk. This becomes prudent with reference to the fact that there is yearly enhancement in payments. This increased income is an outcome of better hygienic and sanitary practices undertaken which ultimately results in better composition of milk (butyrous and protein content). While assessing the sanitary quality bacteriological properties of raw milk, the number of somatic cells, freezing point and quantity of inhibiting substances is considered as measuring points. In cases, where milk quality does not match the foresaid quality standards, the milk gets rejected by the processor wholesomeness.

Conventionally, raw milk is considered to be healthier as compared to the processed milk. This is completely opposite to the fact that raw milk can pose various hazards after consumption. These are correlated with the abundant quantity of micro-organisms in the milk.

Use of plants and plant-based products dates back thousands of years due to the presence of varied micro and macro-nutrients(Lack & Evans, 2021). Balanced diet is a pre-requisite for healthy living. Poor dietary choices pose risks for developing varied disease ailments. Furthermore, a balanced diet is needed for development of neonates into well-built adults.

Researchers have proved that incorporation of plant and plant based products is much needed for healthier living (Pais et al., 2022).

Reforming the dietary patterns and overcoming the conventional food choices is a sort of skill and strategy. Coming up with alternatives is thus wanting.

Milk is considered to be a complete food having ample amounts of calcium, phosphorus, riboflavin, vitamin B12, potassium, vitamin A, zinc, magnesium and proteins (Vranješ et al., 2015). The frequency of milking has no direct correlation with nutritional value of milk. As for now, we have not seen any piece of work establishing any correlation between 3x milking over the quality and total milk yield.

In this paper we tried put forward a foresaid correlation which may be quite beneficial for dairy industry and at large the economics revolving around milk production.

Materials and Methods

Sampling region

The Tissemsilt and Tiaret region of Algeria, which is situated at 16'23" 35° N and 2'19'1° E, is where the samples were collected. With an average elevation of roughly 1080 meters above sea level, 300 to 500 mm of annual rainfall, and an average annual temperature of 22°C, it has a Mediterranean climate. The range of temperatures during the sampling period was 14°C to 28°C. Fig. 1 depicts the research region.

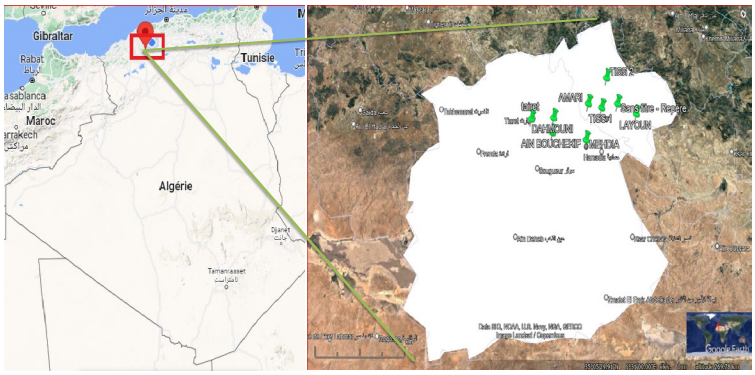

Figure 1. Map of the study.

Dairy farms characteristics and experimental plan

At a schedule of one campaign every 2 months for 144 samples, three sampling campaigns were carried out on 48 farms that were chosen for the study. One milking per day (ODM) or twice a day (TDM) is performed on cows, with the time between milking being as close to one another as is practical. Following homogenization, 200 cc of each sample was taken and placed in glass bottles that had been autoclaved for 20 minutes at 120°C. Additionally, the milk was obtained in accordance with aseptic regulations and Good Laboratory Practices (GLP). The bottles were sent right away to the university lab (Tiaret, Algeria) to cool at 4°C. In order to take account of the real field conditions, no conservative was added.

Physicochemical parameters determination

A lactometer was used to measure the density of milk at 20°C. According to Sarkar et al. (2006), corrected density is equal to density read ± 0.2 times (milk temperature -20°C). According to the manufacturer's instructions, a "Lactoscan" (Milkotronic LTD Europe) was used to evaluate the cow milk's freezing temperature, conductivity, fat and protein levels, lactose concentration, and mineral and vitamin rates (Patbandha et al. 2016).

Microbiological analysis

The International Dairy Federation's guidelines for sample preparation and dilutions for microbiological examination were followed (IDF, 1991).

During the investigation period, every milk sample was submitted to the subsequent microbiological analysis. Total Aerobic Mesophilic Flora (FMAT), Total Coliforms and Fecal Coliforms (CT, CF), Salmonella, Staphylococcus aureus,

Clostridium reducing sulphite, yeasts, and molds are among the microbiological analyses that are carried out. Following a thorough inoculation and a 24-hour incubation period at 30°C, the Total Aerobic Mesophilic Flora (FMAT) is enumerated on the PCA agar. Deep seeding on lactose bile agar with neutral red and crystal violet (VRBL) is used to look for coliforms. (Tir et al., 2015), total coliforms were cultured for 24 hours at 37°C, while fecal coliforms were incubated for 24 hours at 44°C. Surface seeding on solid selective medium (Baird Parker) is used to count Staphylococcus aureus; incubation lasts for 24 hours to 48 hours at 37°C (Hamiroune et al., 2016). Sulfite-lowering The right media (meat and yeast extract medium with sodium sulfite and iron alum) are used to look for Clostridium spores, and they are incubated for 48 hours at 37°C. (Tir et al., 2015). To activate the spores of the clostridium, a 10-minute heat treatment at 80°C is required. 3 steps were used in the search for salmonella: pre-enrichment in buffered peptone water (EPT) for 24 hours at 37°C, enrichment on Bouillon Selinity-Cysteine (SFB) for 24 hours at 37°C, and counting and isolation on Hecktoen medium following a 24-hour incubation period at 37°C. On OGA medium agar, yeasts and molds are enumerated and cultured for 48 hours to 72 hours at 25°C to 30°C. The microbiological data was converted using logarithm base 10 (log) and presented in the descriptive statistic as mean and Standard deviation (Std). The analyses were performed in triplicate.

Data handling and analysis

The TBC, coliform count, and aerobic spore count (mesophilic) findings were converted into base-10 logarithms and represented as CFU/ml and Cell/ml, respectively. An EpiInfodatabase (CDC, version 6.04) was used to enter and maintain the acquired data. After that, descriptive statistics were calculated for each variable. At a critical probability of $p<0.05$, the proportions of continuous and categorical variables were calculated and evaluated for statistical significance using the Chi-square test. SPSS software, version 22, was used for statistical analyses.

Results

Milk yield in ODM and TDM systems

When compared, Results of study showed that ODM system caused a significant decrease in cow production milk by 25% with respect to TDM system.

Physicochemical and nutritional qualities of raw milk in ODM and TDM systems

Furthermore, it was indicated that TDM system influences the pH value of milk. Similarly, average freezing point, density and conductivity are also compromised in TDM as compared to ODM cows. In addition, it was also revealed that as the milking frequency increases the nutritional quality of milk resides. When compared, milk fat, lactose, protein, casein and minerals-vitamins rates were lower by 13%, 15%, 3.9%, 5.3% and 14.4% respectively in TDM group as compared to ODM group.

Microbiological quality of raw milk in ODM and TDM systems

Furthermore, the results revealed that there is a significant difference between the amounts of pathogenic bacterial microbiota in the ODM *vs.* TDM cow groups. Microbiota assessed as TBC, FMAT, TCol and FCol were lowered in ODM by 15.9, 17.9, 19.2 and 23.8 respectively as compared to TDM cows. In addition, the results also suggest that TDM significantly increase the Sulphite Reducing Clostridium (SRC), molds and yeasts count in milk as compared to ODM.

Discussion

A varied number of researchers have revealed that increasing the frequency of milking promotes a rise in milk production, estimated to be between 5% to 10% when compared to a fixed regimen of daily milking (Bogucki et al., 2017; Hogen boom et al., 2019).Several studies have shown that increases milking frequency, favouring an increase in milk yield in the order of 5 to 10% compared with the fixed-frequency regimen of daily milking (Bogucki et al., 2017; Hogen boom et al., 2019).

In our work we observed that usage of TDM increases the milk production by a value of 25% when compared to cows underwent ODM.

Our studies were concurrent with previous published reports. Namely, study by Knight et al. in (1992) postulated that there is an increase by 14% when 4 times milking is compared with 2 times milking groups.

Results of this study demonstrate that the practice of TDM increase significantly the milk yield by 25% as compared to ODM. Similarly, Knight et al. (1992) have reported that milk production increased by 14% after milking 4 times by compared with twice daily milking.

Similarly, Hillerton's study (Hillerton et al. (1990)) explored an increase of 10.4% by increasing the milking frequency.

On the other hand, while reporting their long-term study Klei et al. (1997) reported that the fat yield was alleviated in cows milked thrice by a value of 4.7% as compared to cows milked once per day.

In addition, Hillerton et al. (1990) have demonstrated that increase in milking frequency caused an increase in milk production by 10.4%. In a long-term study, Klei et al. (1997) reported that fat yield produced by cows milked three times daily was 4.7% greater than that from cows milked once daily.

In light of work performed by other researchers, our work is in concordance, as we also evaluated the effect of ODM and TDM on milk physicochemical indices. The acidic index of ODM cows was found to be 6.69 whereas the acidic index of TDM cows was found to be 6.75. These values reside in the range of average pH value. Thus, it can be said that the indices suit the prescribed milk standards.

Results of this study revealed that the acidity of ODM or TDM raw milk is 6.69 and 6.75 respectively. These values samples were moderately acceptable with an average pH of 6, 7. In fact, the samples have a pH outside the fresh milk standards which are prescribed between ranges of 6.6 to 6.8.

Slightly higher range of these indices may an outcome of varied factors. These may range from stage of lactation, including total casein content, presence of mineral ions and salts, as well as hygienic conditions during milking process. Additionally, the overall microbial flora and metabolic activity revolving around it plays a significant role. Lactation can be influenced by various factors including the content of casein, the presence of mineral salts and ions as well as the hygienic conditions during the milking process. Additionally, the overall microbial flora and its metabolic activity may also play a significant role.

In this study, lowering of pH in ODM is asserted to be an after effect of richness of milk with fatty acids (acids thus increased acidity).

This acidity can be natural due to the stage of lactation, the casein content, mineral salts and ion content, or it may be due to hygienic conditions during the milking, the total microbial flora and its metabolic activity (Bousbia et al., 2018; Hameed et al., 2021; Kailasapathy, 2015). The decrease in pH for milk obtained by ODM is probably due to the richness of this milk in fatty acids, vitamins and minerals compared to milk obtained by TDM.

Freezing point is also considered to be an endpoint for assessing the quality of milk. In our study the freezing point of ODM cow milk was estimated to be -0.53°C, which is significantly lower than that of milk obtained by TDM which was found to be -0.74°C.

The average of the freezing point of ODM milk is -0.53°C, which is significantly lower than that of milk obtained by TDM (-0.74°C).

As reported previously it is proved that TDM cow milk is much better than ODM in terms of soluble substances (Ahmad et al., 2013; Dantas et al., 2021; Kailasapathy, 2015).

This shows that milk obtained by ODM is rich in soluble substances such as vitamins, proteins, minerals compared to milk obtained by TDM and therefore better nutritionally (Ahmad et al., 2013; Dantas et al., 2021; Kailasapathy, 2015).

Furthermore, milk density which is a by-product of protein and fat content in milk was also assessed (Ayadi et al., 2009). We observed that the density of milk from ODM cows was 1036 which is much lesser than the milk density of TDM cows i.e. 1025.

In this study, the ODM milk density is 1036, while that of TDM is 1025. this decrease is due to the reduction in milk solids content as protein and fat (Ayadi et al., 2009).

The conductivity values observed ranged from 3.45 µS cm^{-1} to 3.34 µS cm^{-1}, indicating that an increase in milking frequency leads to a reduction in the levels of calcium and other minerals, as demonstrated by our research. In alignment with this Hernández-Castellano et al. (2023) and Loiselle et al (2009) noted that milking four times daily during early lactation diminishes the nutritional quality of milk by lowering nutrient levels. Furthermore, Lacy-Hulbert et al. (1999) found that increasing milking frequency to four times a day during the initial three weeks of lactation enhances milk production while significantly decreasing the concentrations of Calcium, Sodium and Potassium in the milk. The findings of this study indicate that higher milking frequency significantly reduces the composition of milk, including proteins, lipids, casein, vitamins and minerals corroborating earlier observations. In ruminants, plasma glucose levels decline due to the elevated energy requirements associated with milking, as glucose is utilized for lactose production.

The conductivity values ranged from 3.45 µS cm^{-1} to 3.34 µS cm^{-1}, this says that the frequency of milking decreases the rate of calcium and other minerals as showed by our study. Accordingly, Hernández-Castellano et al., (2023) and Loiselle et al., (2009) reported that milking 4 times a day in early lactation reduced the decreases the nutritional quality of milk by reducing the rate of nutrients. Lacy-Hulbert et al., (1999) reported that increasing milking to 4 times a day during the first 3 weeks of lactation increases milk production and decreases significantly the Ca, Na and K levels in milk (Tab. 1).

Table 1. Physico-chemical and nutritional quality of row milk in ODM and TDM systems.

	N	ODM	TDM
Milkyield	40	13.1	9.7
Freezing point (°C)	40	-0.53	-0.74
Density	40	1036	1025
Conductivity (µS cm^{-1})	40	3.45	3.34
pH	40	6.69	6.75
Fat	40	3.31	2.87
Lactose	40	4.17	3.51
Protein	40	2.79	2.68
Casein	40	3.97	3.76
Minerals and Vitamins	40	6.53	5.59

Results of this study showed that the increases of the milking frequency reduced significantly the milk composition as proteins, lipid, casein, vitamins and minerals reflected in findings similar to those described previously. In ruminants, plasma glucose concentrations decrease because of the high energy demands associated with milking and the harnessing of glucose for the production of lactose. Low glucose levels can also be a consequence of decreased appetite and consequently decrease in protein, casein, lipid, vitamin biosynthesis and secretion (O'Brien et al., 2002).The present study clearly showed that the nutritional quality of milk decreased in TDM as compared to ODM; which is in agreement with the findings of previous studies (Hernández-Castellano et al., 2023; Lacy-Hulbert et al., 1999; Loiselle et al., 2009; O'Brien et al., 2002).

Collection and frequency times, storing during and temperature of raw milk from collection until reception in dairy industries are definitely one of the key factors helping to preserve milk quality. The total bacterial count is used as an important indicator of microbial quality of raw milk. The result of microbial quality of raw milk as indicated in tab. 2 shows that total bacterial counts in the ODM were significantly different from TDM.

Table 2. Row milk microbiological quality in ODM and TDM system. Results expressed in log 10cfu/mL Results expressed by Mean ± S.

	ODM			TDM		
	Min	Max	Mean ± SD	Min	Max	Mean ± SD
TBC	4.2	27.3	11.69 ± 0.43	5.9	31.7	13.80 ± 0.74
FMAT (10^5)	2.7	12.4	6.42 ± 0.43	3.1	14.4	7.81 ± 0.43
T.Col (10^5)	0	9.7	4.6 ± 0.41	0	11.3	5.7 ± 0.41
F.Col (10^5)	1.63	7.1	3.29 ± 0.47	1.91	8.7	4.29 ± 0.47
S.R.C	0	73.4	17.1 ± 1.3	0	74.7	19.3 ± 1.3
Molds	0	6.9	3.23 ± 0.33	0	8.1	4.1 ± 0.33
Yeasts	2.1	9.3	4.58 ± 0.29	2.7	10.4	5.22 ± 0.29

Low glucose levels may result from a reduced appetite, which in turn leads to a decline in the synthesis and secretion of proteins, casein, lipids and vitamins (O'Brien et al., 2002). The current study has demonstrated a notable reduction in the nutritional quality of milk from TDM when compared to ODM, aligning with the results of earlier research (Hernández-Castellano et al., 2023; Lacy-Hulbert et al., 1999; Loiselle et al., 2009; O'Brien et al., 2002). The methods of collection, frequency of sampling and the storage conditions, including temperature of raw milk from the point of collection to its reception in dairy processing facilities are critical factors in maintaining milk quality. The total bacterial count serves as a significant measure of the microbial quality of raw milk. As indicated in tab. 2, the microbial quality of raw milk shows that total bacterial counts in ODM were significantly different from those in TDM.

Whereas, we evaluated that the increased frequency of milking is directly proportional to milk contamination. The contamination of milk is usually assessed by the presence of bacterial biota such as TBC, yeast, molds as well as FMAT counts.

In fact, we showed that increasing the frequency of milking increases the risk of contamination of the milk with pathogenic bacteria such as TBC, yeasts, molds, FMAT counts.

Plant and plant-based foods have been considered to be a vital part of one's diet. There are innumerable amounts of nutrients present in plants that enables a being to thrive (Williams & Patel, 2017). Just like milk (animal based product), plant based products as well have an innate quality to provide for an healthier living(Petersen et al., 2018). Just as milk is considered to be a complete provider, plants based diet also maintains a healthy living (Kahleova et al., 2020).

Reducing the frequency of milking effectively minimizes the temperature fluctuations of milk, transitioning from 37˚c during milking to 8˚c during storage, thereby lowering the risk of contamination (Petersen et al., 2018). Previous studies have indicated that milking cow's ones daily allows for increased feeding time compared to those milked twice daily, resulting in milk of superior microbiological quality (Jensen et al., 2023). This observation aligns with earlier findings that suggest an increase in milking frequency leads to a decrease in feeding levels, which subsequently reduces the nutrients available, ultimately diminishing both milk synthesis and quality of milk (Hernandez-Castellano et al., 2023). Furthermore, it has been demonstrated that milk quality is influenced by milking frequency. In dairy cows, as well as in other species, heightened milking frequency under stressful conditions adversely impacts milk quality (Hernandez-Castellano et al., 2023). Additionally, it has been established that milking frequency significantly affects both milk production and quality (van den Borne et al., 2022). Consequently, it can be concluded that milk quality and production are negatively impacted by increased milking frequency to a certain degree.

In fact, reducing milking frequency minimize milk temperature fluctuation from milking (37°C) to storage (8°C) and risk of milk contamination (Reguillo et al., 2018). As previously observed, once-daily milking led to more time spent feeding, compared with cows milked twice daily and consequently milk of good microbiological quality (Jensen et al.,

2023). This was in agreement with the finding of other previous reported whose reports comparatively that increases in milking frequency reduced feeding level reduces the amount of nutrients available to the udder which, in turn, decreases milk synthesis and quality (Hernández-Castellano et al., 2023). This finding agrees with results from were showed that milk quality have been affected by milking frequency. In dairy cows, as in other animals, stressful conditions by increases in milking frequency affect the milk quality (Hernández-Castellano et al., 2023).Whereas milking frequency affect milk production and quality (van den Borne et al., 2022). Therefore, milk quality and production, to a certain extent, is negatively affected by milking frequency.

Once daily milking can help mitigate the negative energy balance that arises immediately after giving birth or during times of energy shortages. It has been also seen that this has a potential to enhance the milk quality up to a certain level.

In fact, once daily milking partially alleviates the negative energy balance that occurs immediately postpartum or during periods of energy deficits and can improve milk quality.

Conclusions

This study emphasises the pivotal influence of milking frequency on the nutritional and microbiological quality of raw milk. The practice of Once-Daily Milking (ODM) has been demonstrated to be an effective method for preserving the nutritional richness of milk while simultaneously reducing the risk of microbial contamination, in comparison to TDM (3 times a day milking). While CT significantly increases milk yield, it is accompanied by a significant reduction in essential nutrients and an increase in the microbial load. These results emphasise the necessity for a strategic equilibrium between productivity and milk quality, while considering the imperatives of sustainability and food safety. Future research should investigate the underlying mechanisms for developing optimal farming practices that reconcile economic efficiency and quality excellence.

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