International Journal of

Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Dairy Science

ISSN 1811-9743
DOI: 10.3923/ijds.2022.71.77

Research Article
Raw Milk Assessment of Dairy Cows Fed with Citronella Distillation Waste Biomass

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Abstract

Background and Objective: Distillation of the Citronella plant (*Cymbopogon nardus* L.) produces essential oils and waste biomass. A preliminary study is needed to explore the relationship between Citronella waste biomass feeding and milk quality. The milk quality from the cows fed with Citronella waste biomass as an alternative forage was assessed in this study. **Materials and Methods:** A total of 10 Friesian Holstein (FH) cows were fed with 40 kg/head/day of solid Citronella distillation waste biomass at 8 am (20 kg/head/day) and 5 pm (20 kg/head/day) for 10 days. The Citronella replaces elephant grass (*Pennisetum purpureum*) as a source of forage. The milk samples were taken compositely on all treated cows and tested for physical quality, proximate analysis, heavy metals and microbial contamination. The analysis results were compared with the raw milk quality of the Indonesian National Standard/Standar Nasional Indonesia (SNI) and other standards from different countries. **Results:** The results showed that the physical characteristics of milk met the SNI. The protein, fat and lactose contents were 3.16 (SNI 2.8%), 5.38 (SNI 3.0%) and 4.63%, respectively. Heavy metal testing showed no mercury (Hg) and arsenic (As), while lead (Pb) was 0.01 g mL^{-1} below SNI (0.02 g mL^{-1}). In addition, no microbial contamination was detected for *Staphylococcus aureus* and Enterobacteriaceae. **Conclusion:** These findings convinced us that Citronella waste could be used as an alternative forage in dairy cattle and produce good quality milk. Further research on the milk quality comparison between Citronella waste feeding and other forage feeding was suggested.

Key words: By-product, essential oil, forage, sustainable, alternative, quality, milk, microbes, contamination, heavy metals, proximate

Citation: Sujianto, S., M.I. Shiddieqy, M. Rizal, S. Sukamto and A. Wahyudi, 2022. Raw milk assessment of dairy cows fed with Citronella distillation waste biomass. Int. J. Dairy Sci., 17: 71-77.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Citronella (*Cymbopogon nardus* L.) is a common grass in South East Asia and surrounding regions. It is mainly grown for its essential oil. Two important compounds, Citronellol and geraniol are used as benchmarks for quality standards which are the basic ingredients for making esters for perfumes and cosmetics. In addition, Citronella oil has antibacterial, anti-fungal and insect repellent functions as a mosquito repellent such as cream and lotion[1,2].

With different extraction techniques, Citronella essential oil's output and chemical composition also changed. The chemical makeup of essential oils varies depending on their geographic distribution[3]. The essential oil of Citronella has been the subject of studies that have identified 100 different components. The major components of the majority of samples were Citronellal, citronellol and geraniol[3]. Growing understanding of the varied qualities shown by essential oils resulted in a dramatic increase in their production and use[3].

The common distillation method used to obtain Citronella oil is a steam distillation system that produces by-products from solid waste[4]. If not utilized further, it can be a critical problem due to voluminous waste that needs space. A non-profitable waste product is created during the distillation of Java Citronella that can be used to potentially make natural antioxidants[5]. The distillation residue of Java Citronella produces a non-profitable waste, which can be valorized as a potential source of natural antioxidants[5]. In searching for value-added efforts in the small distillation industry, distillers utilize about 50% of the distillation waste biomass as additional fuel for the distillation firewood stove[5]. Citronella waste offers a great chance to support ruminant production as a source of fibre feed and a replacement for tropical grass[6].

Utilizing the proper feed processing technique will maximize the nutritional value and digestibility of fibre materials in Citronella waste biomass[6]. Therefore, a model of Citronella distillation and livestock-integrated farming has the potential to be developed. The other example of a successful model has been experimented with using *Aloe vera* waste for improved ruminal fermentation, fibre degradability and decreased methane[3].

The integration system of Citronella and livestock produces Citronella oil and manages agricultural waste as fermented feed for cows and other valuable product[7-9]. However, it has not been assessed scientifically the quality of milk produced. Suppose the nutritional value of Citronella distillation waste biomass is good enough for the cows. In that case, this by-product has the potential to be developed as a forage substitution for livestock. This research aims to determine the quality of dairy cattle fed by Citronella distillation waste biomass.

MATERIALS AND METHODS

Study area: The research was conducted at the Manoko Experimental Station of Indonesia Spices and Medicinal Crops Research Institute at Lembang, West Java, Indonesia. The location of the research station is 60°48'30.75" South Latitude, 107°36'50.68" East Longitude and 1,264 m above sea level. The average annual maximum temperature in Lembang is 28°C, while the average annual minimum temperature is 19°C. The research started in early 2021 for the preparation of animals and Citronella distillation waste biomass. The experiments and data collection were conducted in April, 2021.

Animals: The methods did not generate stress and discomfort for the animals. Therefore, no animals were harmed during the study. The study was conducted on 10 Friesian Holstein (FH) cows. Each cow was fed with 40 kg/head/day of solid Citronella distillation waste biomass at 8 am (20 kg/head/day) and 5 pm (20 kg/head/day). The Citronella feeding replaces Elephant grass (*Pennisetum purpureum*) as a source of forage. The Citronella feeding was conducted for ten days before the milk test was taken. The cows were still given local sources of concentrate feed and formulated concentrate feed from North Bandung Milk Association in addition to forage.

Milk sampling: A sampling of fresh milk was carried out in the morning according to the milking time at intervals of 5.00-6.30 am. The samples were taken compositely on all treated cows and were accommodated in a reservoir bucket, then filtered using a filter cloth. Total filtered milk was put into a special thermos for storing fresh milk for further testing of physical quality, proximate analysis, microbial contamination and heavy metals. The test was conducted at the Bogor Animal Product Quality Testing and Certification Centre.

Milk analysis: The test result was compared with the raw milk quality of the Indonesian National Standard/Standar Nasional Indonesia (SNI) that has been regulated in SNI 3141.1:2011[10] (Table 1). The milk quality can determine the incentive level of the premium price[11]. In addition, the test result was also compared with European Union (EU) standard (Table 2). Milk requirement for the EU follows regulations EC 852/2004 and 853/2004 as a replacement for directive 92/46/EEC[12]. Physical testing includes colour, taste, odour, alcohol test and degree of acidity (pH). The proximate analysis consisted of testing for total solid (%), fat (%), protein (%) and lactose (%). Tests for microbial contamination include total plate count (TPC), *E. coli*, *Coliform*, *Salmonella* sp. and *S. aureus*. Heavy metal testing includes lead (Pb), mercury (Hg) and Arsenic (As).

Table 1: Quality of fresh milk according to Indonesian National Standard/SNI[10]

Characteristics	SNI value[10]
Specific gravity (at temperature 27.5°C) minimum (g mL^{-1})	1.0270
Minimum fat content (%)	3.0
Total solid content without fat (%)	7.8
Minimum protein content (%)	7.8
Minimum lactose content (%)	4.0
Color, odour, taste, viscosity	No change
Potential hydrogen (pH)	6.3-6.8
Alcohol test (70%) (v/v)	Negative
Maximum microbial contamination	
Total plate count (CFU mL^{-1})	1×10^6
Staphylococcus aureus (CFU mL^{-1})	1×10^6
Enterobacteriaceae (CFU mL^{-1})	1×10^3
Total maximum of cell somatic contain (cell mL^{-1})	4×10^5
Antibiotic residue (penicillin, tetracycline, aminoglycoside and macrolide categories)	Negative
Counterfeit milk test	Negative
Freezing point (°C)	-0.520 to 0.56
Peroxidase test	Positive
Heavy metal contamination	
Lead (Pb) (µg mL^{-1})	0.02
Mercury (Hg) (µg mL^{-1})	0.03
Arsenic (As) (µg mL^{-1})	0.1

Table 2: Milk requirements in the European Union (EU) based on directive 92/46/EEC, EC 852/2004 and 853/2004

Characteristics	EU
Basic milk hygiene	
Drugs (µg mL^{-1})	<0.004
Somatic cells (Cell mL^{-1})	400,000
Bacteria (mL^{-1})	100,000
Raw milk for drinking	
Bacteria (CFU mL^{-1})	<20,000
S. aureus (CFU mL^{-1})	<500
Salmonella (CFU mL^{-1})	0
Coliform (CFU mL^{-1})	<100
Pasteurized milk	
Bacteria (CFU mL^{-1})	5,000/50,000
Coliforms (CFU mL^{-1})	<5
Raw milk for production	
Bacteria (CFU mL^{-1})	<100,000
S. aureus (CFU mL^{-1})	<2,000

Statistical analysis: The physical characteristics test used qualitative analysis. Panellists were involved in this test. The proximate test, heavy metals testing and microbial contamination assessment followed standard lab protocols. Descriptive statistics were used to present the lab result.

RESULTS AND DISCUSSION

Nutrition of Citronella waste biomass: Based on previous studies, the nutrition of Citronella waste biomass can be seen in Table 3. The protein content of Citronella waste (7.00%) was higher than that of straw (4.00%). The crude fibre content in Citronella waste was 25.73%, lower than elephant grass and straw, 34.17 and 32.14%, respectively. Meanwhile, the energy content of Citronella waste is 3,353 kcal kg^{-1}, higher than rice straw with a value of 3,167 kcal kg^{-1}.

The availability of quantity protein will change the ratio of carbohydrates fermentability. It is directly related to the microbial biomass in the rumen[15]. In general, feed protein sources are more expensive than feed energy sources. The higher the protein contained, the better the quality of the forage. Therefore, it is necessary to find alternative protein sources that have the same quality as concentrate sources but are affordable to farmers. The main source of protein is usually from the Leguminous family[16]. Suboptimal availability and utilization of Citronella distillation waste allow it to be further formulated as forage.

Physical and proximate test: Expert panellists conduct physical testing visually to distinguish the colour, taste and viscosity of the milk. The observations showed no significant change in milk quality produced from cows fed with Citronella waste compared with other forage. The appearance of milk was a uniform light cream natural colour. It has a sweet taste and a fairly desirable flavour. The alcohol clotting test shows a negative value (normal) or no alcohol formation from the resulting milk. It means there is no alcohol from microbial fermentation due to contamination. Therefore, it meets a standard requirement.

Table 3: Forage and a by-product of Citronella biomass nutrition

Components	By-product Citronella biomass[9]	Elephant grass (75 days after planting)[13]	Rice straw[14]
Protein (%)	7.00		4.00
Fat (%)	2.35	2.01	1.22
Energy (kcal kg^{-1})	3,353		3,167
Crude fibre (%)	25.73	34.17	32.14
Ca (%)	0.35		1.20
P (%)	0.14		1.20
Ash contains (%)	7.91	5.07	22.44

Table 4: Microbial contamination (maximum microbial contamination) on raw milk-fed with Citronella distillation waste biomass

Characteristic	Test result	Indonesian National Standard[10] (maximum)	European Union[12] (maximum)
Total plate count (CFU mL^{-1})	1.5×10^{6}	1×10^{6}	1×10^{5}
Staphylococcus aureus (CFU mL^{-1})	Not detected	1×10^{6}	2×10^{3}
Enterobacteriaceae (CFU mL^{-1})	Not detected	1×10^{3}	-

Finally, the degree of acidity (pH) obtained was 6.8. The current finding for pH was slightly higher than previous research Beni Mellal Region (6.7)[17]. However, it is still in the tolerable range of neutral pH, namely 6.3-6.8 of the Indonesian National Standard. The milk-specific gravity was 1.028, slightly above the normal level (1.027). It was better than a previous study of the quality of raw milk in smallholder farmers conducted by Gwandu *et al.*[18]. Physically, the produced milk is still acceptable to buyers.

The results of the proximate test of cow milk produced from the feed of distilled Citronella were shown in Fig. 1. The test results for the parameters indicated that they were above the quality standard set by Indonesian National Standard[10].

Total solid is one of the main determining factors of price level on milk quality[11]. The total solid result was 11.7%, higher than the minimum standard point (7.8%). The protein content of raw milk shows the milk quality. The higher the protein and fat content, the higher the quality of fresh milk. The content of fresh milk was the main requirement for standard raw materials to be further processed into other value-added products. According to Jørgensen *et al.*[19], consumer interest in high-protein yoghurt has grown, in part due to advancements in flavour and texture. The higher the milk protein content, the better the quality of the yoghurt.

The high-fat, protein and lactose content indicate the large amount of total energy produced from fresh milk. Compared to the other study conducted by Harjanti and Sambodho[20], lactose contains 3.36% and the Indonesian National Standard[10] is 4%. It means the lactose contained in this research was higher than the Indonesian National Standard[10] and previous studies[20].

In addition, the contained essential oil could improve the quality of digestion. Benchaar *et al.*[21] stated that essential oil prospectus improves nutrient digestion efficiency and reduces adverse environmental impact. Furthermore, according to Mishra *et al.*[22], essential oil was a good additive to dairy products, especially for bio-preservative, antioxidant, anti-microbial activities and improving the flavour of the milk.

Microbial contamination level: The results of the microbial contamination test on the milk samples tested were presented in Table 4. The total plate count (TPC) of the milk samples produced by cows fed with Citronella distillation waste feed was slightly higher than the standards set in Indonesian National Standard[10]. It needs attention to improve the way of feeding, the sanitation of the cage and the process of milking the cows.

The microbial contamination contained in fresh milk shows its quality. The microbes, in general, will affect the shelf life and speed of deterioration of milk before it is processed into other products. According to the Center for Food Safety and Food Standards Australia, the total plate count of bacteria in milk ranges from 105 to 107 CFU mL^{-1} [23]. The satisfactory category of milk has a TPC of less than 105 CFU mL^{-1} and the unsatisfactory had a TPC greater than 107 CFU mL^{-1} [23]. It means the maximum microbial contamination was still in the appropriate range. Improvements in sanitation can significantly reduce the acidity level from 0.19-0.14% and reduce the number of bacteria[24].

The high total TPC is usually caused by the lack of sanitation management of the cage. The low amount of TPC in fresh milk may be due to the good pattern of sanitation, cage cleaning, or sanitation of the cage environment[25,26]. Milk that has been exposed to excessive levels of microbial contamination is contaminated with bacteria and cannot be sold to consumers. The TPC levels may be impacted by several aspects of pasteurized milk production[27]. The high levels of microbes were related to increased enzyme activity, resulting in milk and derivative product defects[28]. In addition, raw milk was not contaminated by *Staphylococcus aureus* and Enterobacteriaceae. They are microbes related to human gastroenteritis poisoning and the microbial indicators of hygiene practices and food quality[29-31].

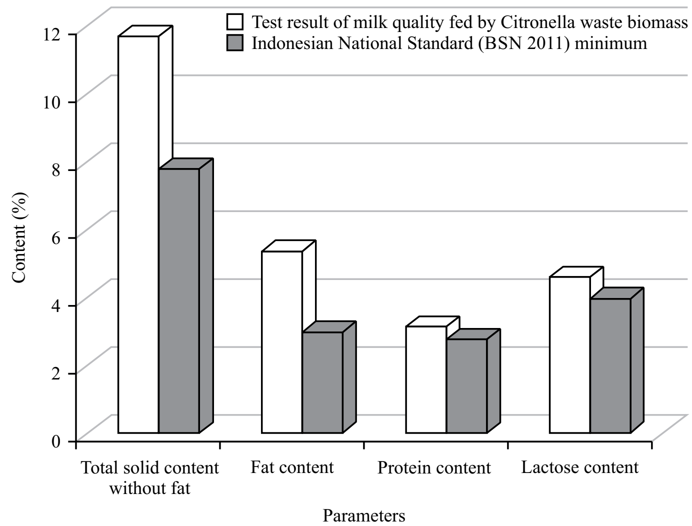

Fig. 1: Proximate levels of raw milk quality from cows fed with Citronella distillation waste biomass

Heavy metal content: The test results on heavy metal contamination showed that the lead (Pb) value of 0.015 μg mL^{-1} was less than the Indonesian National Standard threshold set at 0.02 μg mL^{-1} and a previous study was conducted in Turkey[32]. Meanwhile, mercury (Hg) and arsenic (As) were not detected. Thus, the safety level of fresh milk from cows fed with Citronella distillation waste still meets the established standards.

Citronella distillation waste is a by-product of agriculture, using inputs from fertilizers. It can be exposed to fossil fuel burning or mechanization. The potential risk of heavy metal toxicity in raw milk can be contaminated by environmental pollution and water disposal on the irrigated farm[33-35]. The use of fuel oil containing lead in tractor engines and agricultural vehicles is feared to accumulate in digested forage. Heavy metal contamination can also be obtained from the irrigation system if the water used for cultivation comes from industrial waste.

In this study, preliminary information on the potential of Citronella waste feeding as an alternative forage without lowering the milk quality was provided. This study only compared the milk quality with the national and international standard which make it a limitation of this study. Therefore, further research is needed on milk quality comparison between Citronella waste feeding and forage feeding.

CONCLUSION

The milk quality from dairy cows fed with Citronella distillation waste biomass met the Indonesian National Standard (SNI) for visual tests, proximate analysis, microbial contamination and heavy metal content. The test results showed protein content of 3.16% (SNI 2.8%), fat 5.38% (SNI 3.0%) and lactose 4.63%. Heavy metal testing showed no mercury (Hg) and arsenic (As), while lead (Pb) was 0.01 g mL^{-1} below SNI (0.02 g mL^{-1}). In addition, no microbial contamination was detected for *Staphylococcus aureus* and Enterobacteriaceae. It means that Citronella by-product biomass can be used for alternative forage and does not decrease milk quality.

SIGNIFICANCE STATEMENT

This preliminary study discovered that Citronella distillation waste biomass can be used as forage for dairy cow feed. The milk from cows that fed with Citronella distillation waste biomass met the national standard on physical characteristics and nutritional contents. The milk also passed the heavy metals testing and microbial contamination assessment. This preliminary information will help the researchers to discover other benefits of the Citronella distillation waste biomass as the basis of further research on feed formulation, milk productivity, digestibility, enteric methane emission and comparison with other forages. Distillers produced waste biomass can initiate the utilisation of by-products as added value and promote a circular economy of zero waste concept.

ACKNOWLEDGMENTS

We thank the Indonesian Agency for Agricultural Research Development for providing research funding (SP DIPA 018-092.237306/2021). We also would like to express our gratitude to all technicians at Manoko Experimental Station. We also thank the Animal Product Quality Testing and Certification Center, Bogor, for supporting this research.

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