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EDITORIAL TEAM

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Welcome message from Editor-in-Chief

Dear authors, reviewers, and readers

Our great honor is to present the second issue of the third volume of the Journal of Science and Agricultural Technology (JSAT), the official journal of the Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna (RMUTL), Thailand. The research article still contains five articles from various contributions to this issue. The JSAT has been published in Thai Journal Online (ThaiJO), indexed in Google Scholar, and Digital Object Identifier (DOI) under the National Research Council of Thailand. The journal will publish high-quality articles under an intense peer-review process with solid support from various educational institutions domestically and abroad.

As an Editor-in-Chief, I promise to move forwards to gain international recognition preparing for a higher index ranking. Besides, I strongly encourage researchers around the globe to submit manuscripts to share knowledge and promote the growing field of science and agricultural technology. I am so grateful for the support from our submitting authors, reviewers, and staff. With you, the success of the current issue is possible.

Kind regards,

Assoc. Prof. Dr. Suntorn Wittayakun

Editor-in-Chief Journal of Science and Agricultural Technology
Dean of the Faculty of Science and Agricultural Technology
Rajamangala University of Technology Lanna, Thailand.

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ABOUT THE JOURNAL

Journal of Science and Agricultural Technology (JSAT) publishes original research contributions covering science and agricultural technology such as:

• Natural and applied sciences: biology, chemistry, computer science, physics, material science and related fields. Papers in mathematics and statistics are also welcomed, but should be of an applied nature rather than purely theoretical.

• Agricultural technology: plant science, animal science, aquatic science, food science, biotechnology, applied microbiology, agricultural machinery, agricultural engineering and related fields.

Furthermore, the JSAT journal aims to span the whole range of researches from local to global application.

The JSAT is published two issues a year.
Issue 1: January - June
Issue 2: July - December

Submissions are welcomed from international and Thai institutions. All submissions must be original research not previously published or simultaneously submitted for publication or submitted to other journals. Manuscripts are peer reviewed using the double-blinded review system by at least 3 reviewers before acceptance. There is no publication or processing fee.

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Research Article
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Nutritional, functional, and sensory properties of poundo yam flour enriched with Irish potato flour

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ABSTRACT

This study was conducted to determine the influence of inclusions of Irish potato flour (IPF) into instant poundo yam flour (PYF) on their nutritional composition, functional properties, and sensory evaluation. The four treatments were 1) PYF produced from 100% yam flour (PYF), 2) 90% PYF yam flour + 10% IPF (10IPF), 3) 80% PYF + 20% IPF (20IPF), 4) and 70% PYF + 30% IPF (30IPF). Increasing the concentration of IPF flour increased the concentration of calcium, magnesium, potassium, and iron ($P<0.05$). However, zinc contents were similar ($P>0.05$). For pasting property, 20IPF showed higher peak, trough, breakdown, and final viscosities than 10IPF or 30IPF, but lower than PYF except for trough and final viscosities ($P<0.05$). In contrast, 10IPF showed higher setback viscosity and pasting temperature ($P<0.05$). However, all peak times were similar ($P>0.05$). For functional properties, pH values tended to decrease as the percentage of IPF increased ($P<0.05$); however, loose bulk density, packed bulk density, water holding capacity, swelling capacity, and solubility showed significant increases in response to increases in IPF, except solubility for 10IPF ($P<0.05$). Amylose and carbohydrate values were similar ($P>0.05$). Adding IPF affected amylose content, carbohydrate content, and color properties ($P<0.05$). The finding indicated that enrichment of PYF with IPF benefited mineral contents; 10 to 20 % of IPF might be optimal to include in PYF to maximize overall properties.

Keywords: poundo yam, pasting properties, Irish potato flour, functional properties, sensory evaluation.

INTRODUCTION

Tubers and roots are important carbohydrates as energy sources and are used as staple foods in tropical and sub-tropical countries (Liu et al., 2006). Using tubers as a source of carbohydrate instead of gluten-containing carbohydrates may aid in a reduction in the incidence of celiac disease (CD) or other allergic reactions (Rekha and Padmaja, 2002). Irish potato is an edible tuber from the *Solanum tuberosum* plant, which is actually native to South America, not Ireland. Irish potatoes are named after Ireland because they are closely associated with the Irish potato famine, a historical famine caused by a mold infestation of the Irish potato crop (Mondal et al., 2004; Robert and Cartwright, 2006). Potato production has been considered the first priority compared to other food crops because of its contribution to food security, income generation and double cropping advantages, and its utilization in different forms (Lung'aho et al., 2007; Muthoni and Nyamongo, 2009). It is the fourth most important crop in the world after wheat, maize, and rice, with an annual production of 314.1 million tons cultivated on about 18.1 million hectares of land (Adane et al., 2010).

Yam, a member of the genus *Dioscorea* is the most important staple food in West Africa after cereals (Ekwu et al., 2005). It is a major staple food for an estimated 60 million people in the region stretching from Ivory Coast to Cameroon, an area commonly referred to as the "Yam Zone" of West Africa (Akissoe et al., 2003). Yams are characterized by high moisture content, which renders the tubers more susceptible to microbial attacks and brings about high perishability the tubers. With an annual production of above 28 million metric tonnes (FOS, 2011), Nigeria is the world's largest producer of edible yams, with D. *rotundata* and D. *alata* as the two most cultivated yam species in the country. Yam belongs to the semi-perishable class of food due to its relatively high moisture content and vulnerability to gradual physiological deterioration after harvesting. However, yams can be processed into less perishable products, such as yam flour, through a drying process (Jimoh and Olatidoye, 2009). The traditional process of preparing pounded yam is a tedious process, which involves pounding cooked slices of yam in a mortar using a pestle to create a smooth dough consistency. However, instant poundo yam flour is a modern invention to simplify the tedious traditional process

(Oluwamukomi and Adeyemi, 2015). Instant poundo yam flour requires a short processing time and less energy which benefits preserving yam and reduces human drudgery associated with pounded yam production (Komolafe and Akinoso, 2005). The technology includes peeling, washing, slicing/dicing, cooking, drying, milling and packaging.

There is a need to overcome the challenges of under-nutrition and micronutrient deficiencies through intensive formulation and utilization of locally available food crops (such as yam and Irish potato) as a source of energy, protein, vitamin, and minerals, which will help to fight the problem thus preventing high postharvest losses (Ernest et al., 2017). According to Oluwamukomi and Adeyemi (2015), instant pounded yam flour is a modern invention aimed at simplifying the tedious traditional process of pounding cooked slices of yam in a mortar using a pestle to a smooth dough consistency. As reported by Umadevi et al. (2013), the Irish potato contains pro-vitamin A, vitamin K, sulfur, and a major contributor of vitamin C, thus making it a root crop necessary for consumption. A study conducted by Olumurewa et al. (2019) on production of poundo flour from yam and plantain revealed that the flour performed excellently with satisfactory nutritional and sensory acceptability; thus, a clear indication that poundo yam can also be fortified with other crops. However, there is a paucity of information regarding the fortification of poundo yam with Irish potato flour despite its numerous nutritional benefits; hence, the necessity for this research.

MATERIALS AND METHODS

Source of materials

Yam (*Discorea spp.*) and Irish potatoes (*Solanum tuberosum*) were procured from Owode Market in Offa Local Government Area, Kwara State, Nigeria. Also, Sodium metabisulphite was obtained from the Food processing laboratory of Food Technology Department, Federal Polytechnic Offa Kwara State.

Sample preparation

Preparation of Irish potato flour

The method of Ernest et al. (2017) was used. The Irish potatoes were thoroughly washed, peeled, cut into suitable sizes, and dried in a hot air oven at a temperature of 60oC for 12 hours until a constant weight was obtained. The dried Irish potato samples were subsequently milled into fine particle sizes using an attrition mill and sieved with a sieve with a mesh size of 600 µm to obtain the Irish potato flour (Figure 1).

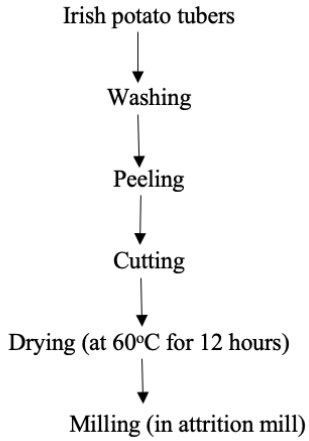

Figure 1. Flow chart for the preparation of Irish potato flour.
Source: Ernest et al., (2017)

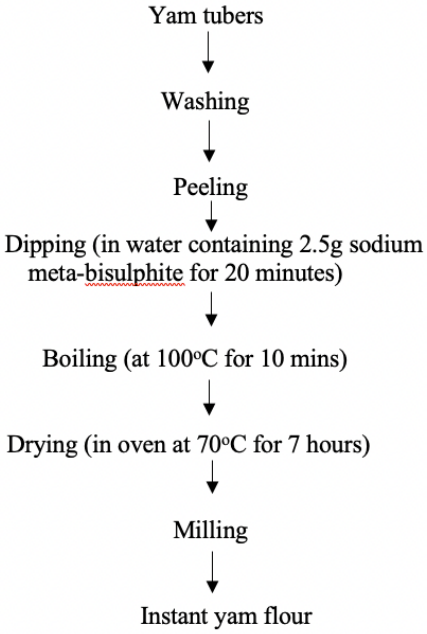

Figure 2. Flow chart for the preparation of instant yam flour.
Source: Oluwamukomi and Adeyemi (2015)

Preparation of instant yam flour

Yam flour was produced according to the modified method of Oluwamukomi and Adeyemi (2015). Briefly, the yam tubers were washed to remove sand, dirt, and other adhering materials. The yam tubers were peeled and sliced to about 0.02 mm thickness, after which they were dipped in water containing 2.5g sodium meta-bisulphite in about 500 ml of water for 20 minutes so as to arrest the browning reaction and placed in a sieve to remove excess water after which they were cooked by boiling

for 10 minutes at 100°C. The cooked (boiled) yams were dried in a hot air oven at 70°C for 7 hours, after which milling using an attrition mill was done (Figure 2).

Treatments

There were four treatments including 1) instant poundo yam flours produced from 100% yam flour (PYF), 2) 90% PYF + 10% Irish potato flour (10IPF), 3) 80% PYF + 20% Irish potato flour (20IPF), 4) and 70% PYF + 30% Irish potato flour (30IPF).

Method of analysis

Mineral determination

1 g of sample was weighed into a digestion tube or a conical flask. 10 ml of H_2SO_4 and 30 ml of nitric acid were added. This was placed on a hot plate in a fume cupboard and digested until the digest became clear. The digest was diluted to 100 ml and taken to Atomic Absorption Spectrophotometer (Buck Scientific Model, 2010, UK), a corresponding lamp for a corresponding mineral was placed in the AAS, and the wavelength specific to a particular mineral to be determined was set. The AAS siphoning hose was dipped into the digested sample after running the standards for the mineral of interest. The concentration of the mineral in the solution was displayed on the screen of the AAS machine (AOAC, 2006).

Determination of pasting properties

A Rapid Visco Analyser, RVA (Model RVA-SUPER3, USA), was used to determine the viscosity of the composite flours according to Hahn and Hozio (1987) method. About 3 g of sample were weighed into a dried empty canister, and then 25 mL of distilled water was dispensed into the canister containing the sample. The suspension was thoroughly mixed so that no lumps were obtained, and the canister was fitted into the Rapid Visco-Analyzer. A paddle was then placed into the canister. The measurement cycle was initiated by depressing the motor tower of the instrument. Samples were pasted according to a programmed heating and cooling cycle. The dispersions were heated from 50 to 95oC with constant stirring at 2.67 Hz, and were held at 95oC for 2.5 min (breakdown). Then, the blocking temperature was cooled to 50oC and held for 2 min. The total cycle was 13 min. Parameters estimated were peak viscosity, setback viscosity, final viscosity, trough, breakdown viscosity, pasting temperature, and time to reach peak viscosity (Hahn and Hozio, 1987.

Determination of pH

A pH meter model (Model PHS-25CW Microprocessor pH/mv meter) was used to determine the pH. 100 ml sterile distilled water was added to ten grams of the flour samples weighed and dissolved in a beaker containing 25ml distilled water to form slurry. It was allowed to stand for 10 min with constant stirring. The pH was then directly determined with the aid of pH meter (Jones et al., 2000).

Water holding capacity (WAC)

1 g of powdered sample was weighed into a previously weighed centrifuge tube. 10 ml of distilled water was added and shaken severally to make sure water circulates throughout the entire powdered sample. The tube and content were centrifuged at 3000 rpm for 30 min, after which the supernatant was decanted. The tube and its content (residue) were weighed. The amount of water absorbed was obtained by subtracting the weight of the tube and sample from the weight of the tube and residue (Onwuka, 2005; Noor Aziah and Komathi, 2009).

Determination of solubility and swelling index/ power

1 g of sample was weighed into a previously weighed empty centrifuge tube. 10 ml of distilled water was added and mixed severally. The tube was placed in a boiling water bath for 30min. After 30 min, the tube was allowed to cool and then centrifuged at 2200 rpm for 15 minutes. The supernatant was decanted into a previously weighed petri dish, and the petri dish was dried in the oven. The tube and its content (gel) were also weighed. The swelling power was calculated by subtracting the weight of the tube from the weight of the tube and gel, while the solubility index was calculated by subtracting the weight of the empty crucible from the weight of the dried crucible and content (residue) (Onwuka, 2005; Noor Aziah and Komathi, 2009; Masur Shakuntala et al., 2009; Kaushal et al., 2012; Jitngarmkusol et al., 2008).

Wettability

The method of AOAC (2006) was used. Into a 25 ml graduated cylinder with a diameter of 1 cm, 1 g of sample was added. A finger was placed over the open end of the cylinder which was invested and clamped at a height of 10cm from the surface of a

600 ml beaker containing 500 ml of distilled water. The finger was removed and the rest material allowed to be dumped. The wettability is the time required for the sample to become completely wet.

Bulk density

This was determined using the method described by AOAC (2006). About 2.5 g of sample was filled in a 10 ml graduated cylinder and its bottom tapped on the laboratory bench until there was no decrease in volume of the sample. The volume was recorded.

$$\text{Bulk density} = \frac{Weight\ of\ sample\ (g)}{Volume\ of\ sample\ (ml)} \qquad \text{equation (1)}$$

Swelling capacity

Swelling capacity was determined according to the method given by Onwuka (2005). About 100 mg of the sample was mixed with 10 ml of distilled water in a calibrated cylinder at room temperature. After equilibration for 18 hours, the bulk volume was recorded and swelling capacity expressed as volume occupied by sample per gram of original sample dry weight.

equation (2)

$$\text{Swelling capacity mL/g} = \frac{Change\ in\ volume\ of\ sample}{Original\ weight\ of\ sample}$$

Determination of colors

This was determined according to Noor Aziah and Komathi (2009) method. 1.00 g of sample was weighed into a beaker and 25 ml ethanol was added. It was stirred for 30 min and allowed to stand for 10 minutes. The supernatant was filtered using filter paper into clean tubes and labeled accordingly. The absorbances of the supernatant were determined using UV visible spectrophotometer at the wavelengths of 615nm, 650nm and 585nm for lightness (l), redness (a) and yellowness (b) respectively. The equivalent values for each colour is obtained (Makanjuola and Coker, 2019; FAO, 2009).

Chemical analysis

Amylose

It was determined by the method of Masur Shakuntala et al. (2009). Iodine reagent was prepared by dissolving 1 g and 10 g potassium iodide in water and making it up to 500 ml mark. 0.1 g of sample was weighed into a flask and 1 ml of distilled ethanol was added followed by addition of 10 ml 1 N NaOH. This was heated for 10 minutes or left over night before continuation. The content was made up to 100ml using distilled water. 2.5ml was taken into a 10ml volumetric flask and 20 ml distill water, followed by addition of 3 drops of phenolphthalein indicator. Few drops of 0.1 N HCl was introduced until the pink colour disappeared. 1 ml of iodine reagent was added and made up to 50 ml with distilled water. The absorbance was read at 590 nm using a spectrum lab23A UV visible spectrophotometer. The concentration was obtained from a standard amylase graph (Bolade et al., 2009; McCready et al., 1950; Juliano, 1971).

Total carbohydrate

Anthrone reagent was prepared by dissolving 0.2g of anthrone powder in 100 ml of 95 % sulphuric acid. 0.1 g of sample was weighed into a centrifuge tube. This was hydrolyzed by adding 5ml of 2.5 N HCl and placing it in a boiling water bath for 3 hours. After 3 hours, it was neutralized by adding solid sodium carbonate until effervescence ceased. The content was transferred into a 100 ml standard flask and made up to mark using distilled water. This was centrifuge,ed and 0.5 ml aliquot was taken for total carbohydrate determination. 4 ml of the prepared anthrone was added and heated in a boiling water bath for 8 minutes. This was co,oled and absorbance read at 630 nm ua sing spectrum 23A UV visible spectrophotometer. The total carbohydrate content was estimated by extrapolating the absorbances from a glucose standard graph (Kaushal et al., 2012).

Statistical analysis

The results obtained from proximate, functional, and pasting analysis were subjected to an independent sample T-test using IBM SPSS (version 20). Significant differences between samples were tested at $P \leq 0.05$ using the Tukey test.

RESULTS AND DISCUSSION

Mineral content evaluation

The mineral component of poundo yam-Irish potato flour produced (Table 1) indicated that calcium content ranged from 18.16 to 35.44 mg/100g. The result showed that 30IPF had the highest value (35.44 mg/100g) while PYF (100% yam flour) had the least value (18.16 mg/100g). The result showed a significant difference between treatments ($P<0.05$). It was observed that the higher the percentage of Irish potato supplementation, the higher the value obtained. The values obtained in all the samples supplemented with Irish potato were significantly higher than PYF. The calcium content obtained in this research was higher than the value (0.47 mg/100g) obtained for 100% yam flour by Thayumanavan and Sadasivam (1984) in effect of water yam and soybean composite flours on the quality of wheat based bread. The variation in the result obtained could be a result of different proportions of IPF used.

Table 1. Mineral contents (mg/100g).

Items	PYF	10IPF	20IPF	30IPF
Calcium	18.16±0.14[a]	23.30±0.76[b]	30.40±0.78[c]	35.44±0.12[d]
Magnesium	51.34±0.11[a]	57.24±0.42[b]	60.91±0.27[c]	65.49±0.18[d]
Potassium	310.35±0.55[a]	352.91±0.31[b]	396.34±0.15[c]	423.92±0.36[d]
Iron	6.23±0.04[a]	6.38±0.09[b]	7.00±0.06[c]	7.59 ±0.16[d]
Zinc	1.10±0.00	1.10±0.00	1.11±0.00	1.13±0.04

IPF = Irish potato flour, PYF = instant poundo yam flour produced from 100% yam flour, 10IPF = 90% PYF + 10% IPF, 20IPF = 80% PYF + 20% IPF, 30IPF = 70% YPF + 30% IPF. Results are mean values of duplicate determination ± standard deviation. Mean values within the same row having different superscripts are significantly different (P<0.05)

Magnesium content ranged from 51.34 to 65.49 mg/100g. The 30IPF had the highest value, while PYF had the least (51.34 mg/100g). The result showed a significant difference between the treatments (P<0.05). The treatment with the highest value would provide the body with the magnesium needed if consumed adequately. This magnesium has been reported to serve as a co-factor in more than 300 enzymes systems that regulate diverse bronchial reactions in the body, including protein synthesis, muscle and heme function, blood glucose control, blood pressure regulation, structural development of bone, nerve impulse conduction, muscle contraction and normal heart rhythm (Sadasivam and Manickman, 2004). Potassium content ranged from 310.35 to 423.92 mg/100g, where PYF had the least (310.35 mg/100g); 30IPF had the highest (423.92 mg/100g) (P<0.05). The results obtained in this work were lower than the result obtained (773.48 mg/100g) for PYF by Thayumanavan and Sadasivam (1984). Potassium influences the contraction of smooth, skeletal, and cardiac muscles and profoundly affects the excitability of nerve tissue. It is also essential in maintaining electrolyte and pH balance (Thayumanavan and Sadasivam, 1984); therefore, consumption of poundo meal supplemented with 30IPF would be of great benefit for people with potassium deficiency. The same trend observed in the aforementioned mineral elements also occurred in iron content of this research work; 30IPF had the highest value of iron (7.59g/100g) while PYF had the least (1.10 mg/100g); however, it was observed that PYF and 10IPF had the same (P>0.05). The increase in supplemented IPF led to the increased value obtained, which could be due to the fact that Irish potato is richer in mineral content than the yam tuber. The result obtained was higher in iron than that reported by Thayumanavan and Sadasivam (1984) obtained (1.84g/100g) for PYF. This will be of nutritional importance, especially to infants and growing children, and pregnant mothers. Sufficient consumption of Iron will help prevent impaired intellectual development in children, lead poisoning in children, anemia in adults and children, and help in the metabolism of almost living organisms and humans. It is an essential component of hundreds of proteins and enzymes (Sadasivam and Manickman, 2004). The result for zinc content showed that 30IPF had the highest value (1.13 mg/100g) while PYF had the lowest (1.10 mg/100g). The result obtained was higher than that obtained (0.70 mg/100g) for PYF by Thayumanavan and Sadasivam (1984). Minerals are important in diets as they play essential roles in body metabolism. For example, calcium helps regulate muscle contractions and transmission of nerve impulses as well as bone and teeth development. Phosphorus has also been reported to be required for bone growth, kidney function, cell growth, and maintaining the body's pH balance (Ochelle et al., 2019). Furthermore, potassium is essential for its vital role is the synthesis of amino acids and proteins. Moreover, magnesium helps in the relaxation of the muscle and the formation of strong bones and teeth. It also plays a fundamental role in most reactions involving phosphate transfer, believed to be essential in the structural stability of nucleic acid and intestinal absorption. At the same time, its deficiency can cause severe diarrhea, hypertension, and stroke.

Pasting properties evaluation

Pasting properties are essential functional characteristics of starches. When an aqueous suspension of starch is heated above a critical temperature, granules swell irreversibly, and amylose leaches out into the aqueous phase, resulting in increased viscosity (pasting). Peak viscosity is a measure of the ability of the starch to form a paste. Starch also can swell freely before its physical breakdown (Fallon and Enig, 2001). Peak viscosity has been reported to be closely associated with the degree of starch damage. According to Fallon and Enig (2001), high starch damage results in increased viscosity. The result obtained in this research work (Table 2) showed that PYF had the highest value (1728.50 RVU) while 10IPF had the least (1377.40 RVU) (P<0.05). The peak viscosity obtained for this research work was higher than the result obtained by Sanni et al. (2001), who reported peak viscosity ranging from 325-398 RVU for starch extracted from sorghum; however, it was low when compared with the result obtained by Aviara et al. (2010), who recorded peak viscosity of 639-726 RVU for yam starches. The higher peak viscosity observed in 100% yam flour may be suitable for products requiring high gel strength, thick paste, and elasticity.

Table 2. Pasting properties.

Items	PYF	10IPF	20IPF	30IPF
Peak (RVU)	1728.50±0.71^{d}	1377.10±1.98^{a}	1627.51±3.52^{c}	1483.50±3.54^{b}
Trough (RVU)	922.04±1.47^{c}	812.51±2.14^{a}	928.00±2.83^{c}	821.00±4.24^{b}
Breakdown (RVU)	808.01±1.42^{d}	567.50±3.54^{b}	697.50±3.54^{c}	654.50±3.54^{b}
Final viscosity (RVU)	1744.55±5.02^{a}	1790.02±0.28^{b}	1852.51±7.77^{d}	1811.50 ±3.54^{c}
Setback (RVU)	821.00±1.41^{a}	980.50±2.12^{c}	929.00±1.41^{b}	965.00±35.34^{bc}
Peak time (min)	5.70±0.14	5.35±0.64	5.85±0.28	5.71±0.35
Pasting temp (^{0}C)	80.87±0.05^{a}	89.45±0.21^{c}	82.13±0.18^{a}	85.75±2.12^{b}

IPF = Irish potato flour, PYF = instant poundo yam flour produced from 100% yam flour, 10IPF = 90% PYF + 10% IPF, 20IPF = 80% PYF + 20% IPF, 30IPF = 70% YPF + 30% IPF. Results are mean values of duplicate determination ± standard deviation. Mean values within the same row having different superscripts are significantly different (P<0.05).

The trough (Table 2) ranged 812.51 to 928.00 RVU; 20IPF had the highest value (928.00 RVU) while 10IPF had the least (812.51 RVU) (P<0.05), but there was no significant difference between PYF and 20IPF (P>0.05). These results were lower than the result reported (1688 RVU) by Olumurewa et al. (2019), but higher than those (38.040 to 262.830 RVU) reported by Amoo et al. (2014). The holding period (Trough) sometimes referred to as shear thinning, holding strength, or hot-paste viscosity is a period when the samples were subjected to a period of constant temperature and mechanical shear stress. Breakdown results ranged from 567.50 to 808.01 RVU (P<0.05); PYF had the highest value (808.01 RVU) while 10 IPF was the least (567.50 RVU). Breakdown measures the ability of starch to withstand collapse during cooling or the degree of disintegration of granules or paste stability (Oduro et al., 2001). Adebowale et al. (2005) reported that the higher the breakdown viscosity, the lower the ability of the sample to withstand heating and shear stress during cooking. The breakdown viscosity of this research work was higher than the range obtained by Sanni et al. (2001) (145 to 216 RVU) for sorghum and (15 to 385 RVU) for yam starches (Aviara et al., 2010). The result showed that sample 10IPF would withstand heating and shear stress during cooking more than other treatments. Setback value ranged from 821.00 to 980.50 RVU. Setback measures the re-association of starch (Jimoh et al., 2009). High setback value is associated with cohesive paste. The result obtained from this work (821 to 980 RVU) was higher than the values obtained by Sanni et al. (2001) and also higher than the value obtained by Aviara et al. (2010) for yam (79 to 339 RVU). Low setback values are useful for products like weaning foods which require low viscosity and paste stability at low temperature (Adebowale et al., 2005). Hence, the Irish potato may be useful for such products. Conversely, starch from Irish potato may be useful for products such as pounded yam that require high cohesive pastes. The final viscosity ranged from 1744.55 RVU for PYF to 1852.51 RVU for 20IPF where the highest value was 20IPF and the least value was PYF. The final viscosity of 20IPF (1852.51 RVU) indicated the ability to form a firm, viscoelastic paste or gel after cooking and cooling owing to the re-association of starch molecules. This work was not aligned with the finding of Amoo et al. (2014) who reported high values ranged value of (84.04 to 356.79 RVU) for the various yam starches produced. The result obtained was within the range (1409 to 2430 RVU) and in agreement with reported by Olumurewa et al. (2019) in the evaluation of functional and pasting properties of instant pounded yam/plantain flour. The pasting time of this research work ranged from 5.35 to 5.85 minutes (P>0.05). The pasting time of the composite flour used in this research work is low compared with the pasting time recorded by Aviara et al. (2010), who observed pasting time of 17.40 to 17.55 minutes for yam varieties. The slight difference in the pasting time may be attributed to a difference in formulation. The starches with a shorter pasting time such as that of 10IPF may be appropriate for the production of foods that require shorter processing time. It can be observed from the results (Table 2) that the higher the pasting temperature, the shorter the pasting time. The pasting temperature provides an indication of the minimum temperature required for sample cooking, energy cost involved and other component stability (Jimoh et al., 2009). It also gives an indication of the gelatinization time during processing (Ikegwu et al., 2009). The associative bonding of the amylose fraction is responsible for the structure and pasting property of the starch granule. The pasting temperature in this research work ranged between (80.87^{0}C - 89.45^{0}C). The result showed no significant difference between PYF and 20IPF (P>0.05). The pasting temperature of the yam-Irish potato flour used in this work is greater than the results obtained by Aviara et al. (2010), who recorded pasting temperatures for starches obtained from 4 local yam varieties, which ranged from 75.1 to 77.3°C.

Functional properties evaluation

Functional properties of flour products are among the most important parameters used to ascertain the suitability of flour and starch for certain end uses. Afoakwa et al. (2021) stated that functional properties aid in the choice of a variety for use in the industry as a thickener, binder, or any other food and

industrial use. According to Afoakwa et al. (2021), functional properties are the properties of a food product that provide information on how food ingredients behave in a food system during processing. These properties include water absorption capacity, solubility, swelling power/index, wettability, bulk density, swelling capacity/volume, and gelation properties. These properties regulate the sensory characteristics and stability of processed starch products. Many factors have been stated to impact the degree and type of functional properties of foods. These factors, according to Mégnanou et al. (2009), including the starch composition and concentration, the ratio of amylose to amylopectin, characteristics of each fraction in terms of molecular weight/distribution, degree/length of branching, and conformation of starch.

The pH of poundo yam-Irish potato flour supplementation (Table 3) is important because it affects most of the functional properties of the flour (Odedeji and Adeleke, 2010). The pH values ranged from 5.43 to 5.71 ($P<0.05$). A decrease in pH was observed along with increasing in IPF percentage ($P<0.05$); however, the pH values of 10IPF and 20IPF were similar ($P>0.05$). PYF had the highest pH (5.71) while 30IPF had the least value (5.43). Low pH values have been reported to be caused by high amylase activity which increases the level of acidity. The pH will affect palatability and discourage the growth of pathogenic bacteria and subsequent spoilage of the yam flour (Eleazu and Ironua, 2013). pH is also a critical factor that influences the reconstitution characteristics of food products. Loose bulk density (Table 3) ranged from 0.48 to 0.52g/mL. PYF had the lowest value (0.48g/mL) while sample 30IPF had the highest value (0.52g/mL). There was no significant difference between 10IPF and 20IPF ($P>0.05$). Packed bulk density ranged from 0.73 to 0.80g/mL ($P<0.05$); 30IPF was the highest in packed bulk density (0.80g/mL) while PYF was the lowest (0.73g/mL). It was observed that the packed bulk density increased in response to increasing IPF supplementation. The result obtained for both bulk density in this research work was slightly lower than the result (0.84g/mL) recorded by Eleazu and Ironua, et al. (2013) for yam flour in rheological and functional properties of soy-poundo yam flour. The bulk density is influenced by particle size and the density of the flour and is important in determining the packaging requirement and material handling. Bulk density is influenced by the structure of the starch polymers, and the loose structure of the starch polymers could result in low bulk density. The high loose and packed bulk density indicated its heaviness, this means that 30% Irish potato supplemented might be useful in food preparations to reduce paste thickness in food products, as well as in the pharmaceutical industry as a drug binder and disintegrant (Solakunmi et al., 2013; Chandra et al., 2015). The results of bulk density revealed that it depended on the particle size and initial moisture content of flours (Zaku et al., 2009). The increase observed in this study in bulk density was not desirable in packaging because higher density often results in reduced ability to compress the flour. This is unlikely to result in cost savings since more packaging materials would be required.

Table 3. Functional properties.

Items	PYF	10IPF	20IPF	30IPF
pH	5.71±0.01^{c}	5.58±0.00^{b}	5.53±0.03^{b}	5.43±0.04^{a}
Loose bulk density (g/mL)	0.48±0.01^{a}	0.49±0.00^{b}	0.50±0.00^{b}	0.52±0.01^{c}
Packed bulk density (g/mL)	0.73±0.00^{a}	0.74±0.00^{b}	0.79±0.00^{c}	0.80±0.00^{d}
Water holding capacity (%)	342.30±0.65^{a}	359.14±0.23^{b}	374.41±0.18^{c}	386.03 ±0.34^{d}
Swelling capacity (ml/g)	620.65±0.50^{a}	634.01±0.23^{b}	683.64±0.19^{c}	687.90±0.34^{d}
Solubility (%)	5.07±0.05^{b}	3.79±0.02^{a}	7.37±0.13^{c}	9.16±0.08^{d}

IPF = Irish potato flour, PYF = instant poundo yam flour produced from 100% yam flour, 10IPF = 90% PYF + 10% IPF, 20IPF = 80% PYF + 20% IPF, 30IPF = 70% YPF + 30% IPF. Results are mean values of duplicate determination ± standard deviation. Mean values within the same row having different superscripts are significantly different ($P<0.05$).

The water absorption/holding capacity (Table 3) of poundo yam flour ranged 342.30 to 386.03 %. The highest value was recorded for 30IPF (386.03 %) while PYF had the least value (342.30%) ($P<0.05$). The result obtained in this research work was high compared to the result reported (2.70 %) by Ezeama (2007) for flour in rheological and functional properties of soy-poundo yam flour. Water absorption capacity influenced product viscosity and the properties of starch system (Chandra and Singh, 2013). High water holding capacity may assure product moisture stability (Richana and Sunarti, 2004). Water absorption capacity is useful in determining the capacity of flour to take up water and swelling to improve uniformity in food. It is also advantageous in food processing for improving yield, uniformity, and giving shape to food products (Adebowale et al., 2007); the higher value of water holding capacity may cause by a high polar amino acid residue of protein having an affinity for water molecule (Osundahunsi et al., 2003). Since they have hydrophilic parts such as polar or charged side

chains, proteins and carbohydrates are the major chemical constituents that increase the water holding capacity of flours. The water holding capacity of the flours could also be influenced by an increase in the solubility, leaching out of amylose, and loss of molecular structure of the starch as well as the crystalline structure (Yusuf et al., 2008). It might also be influenced by the non-identical structure of flours. The high water holding capacity of the flours showed that they could be used in the formulation of various foods such as meat sausage, bakery products, dough, and processed cheese (Hashimoto and Grossman, 2003).

The swelling capacity ranged from 620.65 to 687.90 ml/g. The highest swelling capacity was recorded for 30IPF (687.90 ml/g) while PYF had the least value (620.65 ml/g). It was observed that the higher the Irish potato supplementation, the higher the value recorded (P<0.05). The result obtained in this research work was higher than the range value recorded (9.70 to10.20 ml/g) by Amoo et al. (2014) in the evaluation of starch from trifoliate yam (*Dioscorea dumetorum*) Landraces. The swelling power is an indication of the presence of amylase which influences the quantity of amylase and amylopectin present in the yam flour. The swelling power of flour granules is an indication of the extent of associative forces within the granule. Swelling power is also related to the water absorption index of the starch-based flour during heating. Therefore, the higher the swelling power, the higher the associate forces. The variation in the swelling power indicates the degree of exposure of the internal structure of the starch present in the flour to the action of water (Malomo et al., 2012).

The solubility index ranged from 3.79 to 9.16 % (P<0.05). 10IPF had the lowest value (3.79%) while 30IPF had the highest value (9.16 %). The result obtained in this research work was lower than the result recorded (16.16 %) for yam flour by Udensi and Okaka (2000) in Rheological and Functional Properties of Soy-Poundo Yam Flour. The wettability capacity values ranged from 32.50 to 59.50 sec; wettability value was highest for 30IPF with a value of 59.50 sec while 10IPF had the least value at 32.00 sec. There was no significant difference between PYF, 10IPF, and 20IPF (P>0.05). These wettability capacity values were higher than the value of 42.5 sec reported for *D. rotundata* (Udensi and Okaka, 2000) and 27 to 35 sec for *D.alata* flours (Udensi et al., 2008) of similar products, but lower than 135 to 148 sec for soy-melon 'garri' (Oluwamukomi and Jolayemi, 2012). This means these flours were denser and would sink in water more than those reported. Wettability provides a useful indication of the degree to which dried flour is likely to possess instant characteristics which are aided by high porosity.

Amylose and carbohydrate evaluation

Amylose contents (Table 4) ranged from 13.00 to 19.47%. The results showed significant differences among treatments (P<0.05). PYF had the highest value (19.47%) while 30IPF had the least value (13.00%). It was observed that the higher the supplemented sample the lower the value obtained. The result obtained in this research work was lower to the result recorded (35.92%) for yam flour by Oluwamukomi and Akinsola (2015) in thermal and physicochemical properties of some starchy foods: yam (*Dioscorea rotundata*), cocoyam (*Xanthosoma sagittifolium*) and plantain (*Musa paradisiaca*). The amylose content is simply the linear molecular structure of starch. It is an important factor with regard to the end use properties of various products such as noodles and dough. It has a strong bond and therefore takes a lot of energy to breakdown during digestion due to its tightly packed structure. It is reported to be an effective prebiotic. Amylose positively influences the functioning of the digestive tract microbial flora, the blood cholesterol level and the glycemic index, and assists in the control of diabetes (Hu et al., 2012).

The total carbohydrate ranged from 90.06 to 93.09%; PYF had the highest value (93.09%) while 30IPF had the least (90.06%) (P<0.05), but there was no significant difference between 10IPF and 20IPF (P>0.05). The high carbohydrate value in YPF could be attributed to the high content of starch in yam tuber.

Table 4. Amylose and carbohydrate contents (%).

Items	PYF	10IPF	20IPF	30IPF
Amylose	19.47±0.72^{d}	17.61±0.21^{c}	15.03±0.11^{b}	13.00±0.18^{a}
Carbohydrate	93.09±0.48^{c}	90.66±0.15a^{b}	91.45±0.28^{b}	90.06±0.59^{a}

IPF = Irish potato flour, PYF = instant poundo yam flour produced from 100% yam flour, 10IPF = 90% PYF + 10% IPF, 20IPF = 80% PYF + 20% IPF, 30IPF = 70% YPF + 30% IPF. Results are mean values of duplicate determination ± standard deviation. Mean values within the same row having different superscripts are significantly different (P<0.05).

Color property evaluation

The results of color measurement, as presented in Table 5, were significantly (P<0.05) different in lightness (L^*), redness (a^*), and yellowness (b^*) values for all the flour treatments. Lightness values ranged from 60.85 to 84.21; PYF had the highest value (84.21), while 30IPF had the least (60.85) (P<0.05). Yellowness result showed that 30IPF had the highest value (15.17) while YF sample had the lowest value (5.85) (P<0.05). High yellowness values in supplemented samples could be due to the higher amount of beta carotene in supplemented flour (Ahmed et al., 2005). Beta-carotene is a very sensitive nutrient that degrades during processing or storage and occurs via oxidation or isomeration (Van Hal, 2000). Irish potato flour could add natural color to food products. Results obtained for the measurement of redness represented by a^* values indicated that redness (a^*) was significant (P<0.05) and showed an increase in redness values which ranged from 1.02-2.47 as supplemented increased. A higher redness value of 2.47 was obtained in 30% Irish potato flour. The higher redness value could be due to the presence of the anthocyanin pigments in the Irish flour.

Table 5. Color properties.

Items	PYF	10IPF	20IPF	30IPF
Lightness (L^*)	84.21±0.16^d	79.55±0.18^c	67.07±0.13^b	60.85±0.49^a
Yellowness (b^*)	5.85±0.28^a	8.22±0.16^b	11.66±0.64^c	15.17±0.52^d
Redness (a^*)	1.02±0.85^a	1.77±0.02^b	2.10±0.07^c	2.47±0.50^d

IPF = Irish potato flour, PYF = instant poundo yam flour produced from 100% yam flour, 10IPF = 90% PYF + 10% IPF, 20IPF = 80% PYF + 20% IPF, 30IPF = 70% YPF + 30% IPF. Results are mean values of duplicate determination ± standard deviation. Mean values within the same row having different superscripts are significantly different (P<0.05).

Sensory evaluation

Sensory evaluation of poundo-yam-Irish potato (Table 6) produced showed the preference of the panelists for the poundo yam produced. For color, PYF tended to obtain a higher value at 8.5 while 30IPF the lowest at 6.5. The preference for the color decreases as the substitution with IPF increases this may be due to the fact that consumers are already used to the off-white or creamy color of PYF. For aroma, PYF tended to have the highest value (8.2) while 30IPF had the least value (6.1%). For taste, 30IPF tended to have the least value (5.9) while PYF tended to obtain the highest value (8.5). For appearance, PYF tended to be the most preferred while 30IPF was the least preferred. For texture, PYF had the highest value (8.6) while 30IPF had the least value (5.8). For overall acceptability, sample PYF was most preferred by the panelist (8.7) while 30IPF was least preferred (6.4). All the samples were acceptable by the panelist meanwhile PYF was rated the highest. Consumers' preference for PYF could be attributed to their familiarity with the product more so, the pounded yam for which the poundo flour is an alternative is originally made from yam only.

Table 6. Sensory evaluation.

Items	PYF	10IPF	20IPF	30IPF
Color	8.5	7.5	7.5	6.5
Aroma	8.2	7.4	7.4	6.1
Taste	8.5	7.2	7.9	5.9
Appearance	8.5	7.4	7.9	5.7
Texture	8.6	7.7	7.4	5.8
Overall acceptability	8.7	7.6	7.1	6.4

IPF = Irish potato flour, PYF = instant poundo yam flour produced from 100% yam flour, 10IPF = 90% PYF + 10% IPF, 20IPF = 80% PYF + 20% IPF, 30IPF = 70% YPF + 30% IPF. Results are mean values of duplicate.

CONCLUSIONS

The research revealed the mineral content and pasting properties of PYF-IPF. For mineral content, there was an increase in values obtained as the percentage of supplemented IPF increased while PYF had the least value. Functional and pasting properties showed that all the treatments could be acceptable for industrial use. However, 10 to 20 % of Irish potato flour may be an optimal dose added into instant poundo yam flour to achieve optimal responses to overall property criteria. In sensory evaluation, the inclusion of IPF tended to decrease acceptability of consummers.

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Utilization of *Carissa carandas* Linn. aqueous extracts as reducing agent for traditional cotton fabrics dyeing with indigo from *Strobilanthes cusia* Nees

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ABSTRACT

This current study investigated the aqueous extracts of *Amaranthus viridis* and *Carissa carandas*as reducing agents in indigo dyeing fabric with *Strobilanthes cusia* compared to the traditional plant *Tamarindus indica*. Freshly prepared extracts were subjected to preliminary phytochemical screening. It was found that *C. carandas* extract revealed the presence of pH 2.6 and the content of phenolic, flavonoid, and tannin of 0.93 GAE/mg DW, 0.92 QE/mg DW, and 7.55 ECGC/mg DW, respectively. This might be responsible for the reduced properties. The reducing power measured by DPPH assay and ferric ion reducing antioxidant power (FRAP) confirmed that 10% (w/v) of *C. carandas* extract possessed the excellent property closely to *T. indica* extract, whereas 15%-20% (w/v) *C. carandas* extract with 10% (w/v) bio-mordant was found to contain the closely colorimetric characteristics (CIE L*a*b*) to *T. indica* extract. It can be concluded that *C. carandas* extract has high potential as a reducing agent in fabric dyeing with *S. cusia* and might be a promising low-cost reducing agent for developing green dyeing for clothing in the near future.

Keywords: *Carissa carandas*, cotton fabrics, dyeing, reducing agent, indigo

INTRODUCTION

Cotton fabric dyeing with natural dyes is an important culture of the northern people in Thailand, including Chiang Mai, Phrae, Nan, and Lampang provinces. The fabulous dyes obtained from plants such as *Strobilanthes cusia* Nees (indigo plant) have been used for many decades to meet the community way, customer requirements, and serve the conservative trend of natural products. This variety of indigo plants is widely found in the northern region of Thailand. These dues to the suitable weather, which is colder and has a higher moisture content than the other parts of Thailand. For this reason, this type of indigo plant makes the indigo-dyed fabrics of northern Thai more charming and unique.

However, the critical steps of conventional dyeing, such as water-to-material ratio, fermentation time, fermentation temperature, lime quality, pH, and dissolved oxygen concentration, are influent related to indigo yield and qualities (Pattanaik et al., 2021; Li et al., 2019). In the traditional method of indigo dyeing preparation, indigo leaves were immersed in lime hydrate, $Ca(OH)_2$, in water and thoroughly stirred for 48 hours at ambient temperature to extract pigment indigo. The supernatant and debris were removed, and precipitated indigo extracted (Hom-Peag) was collected. Afterward, the insoluble extract was reduced into leuco-white form, more solubilized using the reducing agent tamarind (*Tamarindus indica*). Unfortunately, tamarind costs constantly fluctuate in the rainy season due to fungal disease and rain deficit during peak flower and fruit-bearing stages. Therefore, two new reducing agents in the local area were investigated using equivalent tamarind properties for the *S. cusia* dyeing process.

Amaranthus viridis (Thai; Pak-Khom), an annual herb in the family *Amaranth ceae*, is widely grown and consumed in northern and northeastern Thailand at a low cost. It was reported that total phenolic contents, total flavonoid contents, tannins, saponins, alkaloids, and glycoside derivatives were rich in leaves. These components contributed to reducing properties measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reducing ability assay (Ahmed et al.,2013). Thus, it might be the new challenged plant for use in this experiment as a reducing agent. Another material such as *Carissa carandas* Linn. is also documented as a reducing agent by Khunchalee and Charoenboon (2019). Its extract possessed a high content of total phenolic contents which contributed to high antioxidant properties evaluated by using DPPH, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Ferric reducing antioxidant power (FRAP) assay.

Furthermore, the *Carissa carandas* Linn was found abundantly in northern Thailand as an ornamental plant with its low-cost fruit.

In this work, these local plants were taken for phytochemicals investigation and used in traditional cotton fabrics dyeing as reducing agents with *Strobilanthes cusia* Nees.

MATERIALS AND METHODS

Dye extraction from aerial parts of indigo plant

One kilogram of aerial parts from the indigo plant was chopped, immersed in 10 L of distilled water, and naturally fermented for 24 hours at ambient temperature. Afterward, the residual was removed, and 120 g of $Ca(OH)_2$ was added to a solution. Then, the solution was mixed vigorously to oxidize the mixture for 20-30 minutes or until the solution turned to blue color. Afterward, the supernatant was discarded by using cheesecloth, and the dye paste was collected for further studies.

Indigo dye solution preparation

Sixty grams of dye paste and 30 g of $Ca(OH)_2$ were mixed and dissolved in 1.4 L of water. The mixture was boiled at a controlled temperature of 40°C with gentle and continuous stirring. After heating for 10 min, the 250 mL of 10% reducing solution agent was added and gently stirred for 40 min to remove oxygen. When the foam decreased, the solution turned yellowish green, and the foam color was bluish purple.

Reducing solution agent preparation

As a traditional method, the 100g of *Tamarindus indica* (TI-) extract was prepared in 1 L of hot water (90 °C). The samples, *Carissa carandas* Linn. (CC-) and *Amaranthus viridis* (AV-) extracts, were also conducted similarly.

Characterization of reducing agent

The UV-Vis spectral analysis of the reducing agent was conducted using a UV-Vis spectrophotometer in the range of 200-1000 nm.

Determination of reducing capacity

DPPH assay

The reducing ability of antioxidants toward the DPPH radical was measured in triplicates, according to Yodthong et al.(2020a). The purple DPPH working solution (oxidized form) was prepared in absolute ethanol to achieve the final absorbance of 1.0 ± 0.2 at 495 nm measured using a microplate reader (BIOBASE, China). Ten microliters of appropriately diluted sample were mixed with 200 µL of the working solution. After incubation for 30 min at the ambient temperature in the dark in the microplate, the yellow oxidized form DPPH was generated; then, the absorbance was determined at 495 nm using a microplate reader. The percentage of inhibition of reducing ability was calculated using the equation:

$$\% \text{ Inhibition} = [(OD_{max} - OD_{sample})/OD_{max}] \times 100$$

The linear curve was generated by plotting the percentage of inhibition against the concentration, and the results were expressed in microgram/mL (R^2 = 0999). After the trend line was plotted with the regression equation in Microsoft Excel 2019 software, put y=50, and calculated the x value in appropriated dilution to get the IC_{50} of DPPH.

Ferric reducing antioxidant power assay

FRAP assay was performed in triplicated according to the protocol to 96-well microplates of Carrasco-Sandoval et al. (2022) with some modifications. FRAP working solution consisted of 0.3 M of acetate buffer (pH 3.6), 0.01 M of HCl (Labscan, Thailand), and 0.02 M of $FeCl_3 \cdot 6H_2O$ (Merck, Germany) in the ratio of 10:1:1 (v/v). Twenty microliters of appropriately diluted sample were mixed with 280 µL of working solution. After incubation for 30 min at the ambient temperature in the dark in the microplate, the tripyridyltriazine complex was formed; then, the absorbance was determined at 630 nm by using a microplate reader (Biobase EL-10A, China), and the results were expressed in microgram Gallic acid equivalent (GE) per g of dry weight (DW).

Determination of total phenolic content

As indicated by Sassa-deepaeng et al. (2019), the total phenolic content (TPC) was determined by using Folin-Ciocalteu (FC) colorimetric method in triplicates. Twenty µL of TI-, CC-, or AV- extracts were shaken rigorously with 100 µL of FC (Merck, Germany) reagent in 1,980 µL of DI water. After incubation for 5 min at ambient temperature, 300 µL of 7% Na_2CO_3 (Univar, Ajax Finechem, Australia). The mixtures were incubated in the dark for 60 minutes before measurement at 765 nm using a spectrophotometer. The content of TPC was expressed as microgram gallic acid equivalent (GAE) per milligram dry weight.

Determination of flavonoid content

The flavonoid content (FC) was examined by the aluminum trichloride ($AlCl_3$) colorimetric method with some modifications by Yodthong et al.(2020b) in triplicates. Twenty-five µL of various

extract concentrations was added to 75 µL of DI water followed by 25 µL of 5% $NaNO_2$ (Univar, Ajax Finechem, Australia) on a microplate and incubated for 5 min at the ambient temperature. Afterward, the mixture was mixed with 25 µL of 10% $AlCl_3$ (Lobachemie, India) and then incubated for 6 minutes under the same condition. At the final step, 100 µL of 1M NaOH (Merck KGaA, Germany) was added and then incubated for 30 min in the dark. The mixtures were incubated again in the dark for 60 minutes before measurement at 405 nm using a microplate reader. The flavonoid content was expressed as microgram quercetin (Sigma–Aldrich, Germany) equivalent (QE) per milligram dry weight.

Determination of tannin

The content of tannin affected the reducing properties of the sample. To measure the tannin content (TC), the vanillin assay was performed in triplicates according to the protocol of Sassa-deepaeng and Yodthong (2019). The 250 µL of the extract was mixed with 450 µL of 1% of vanillin (Merck, Germany) reagent and incubated for 5 min at the ambient temperature in the dark. Afterward, the mixture was shaken with 300 µL of concentrated HCl (Labscan, Thailand) and then incubated for 30 minutes under the same condition. Finally, the color of the solution turned red, and the absorbance was measured at 500 nm. The tannin content was expressed as microgram Epigallocatechin gallate (Sigma–Aldrich, Germany) equivalent (EE) per milligram dried weight.

Dyeing

The clothing samples with the dimension of 10×10 cm (W×D) were prepared. Pre-dyeing was conducted by washing the fabrics with detergent and water to remove coated starch. Damp fabrics were dyed in a working solution for 2 min at ambient temperature and then exposed to oxygen for 2 min. After the oxygenation process, the dyed fabrics turned blue. To intensify the fabrics, the dyeing was repeated five times. The dyed cloth samples were washed in water, twisted tightly until damp, and soaked in warmed 10% tea solution (60°C) for 2 min. The samples were then washed and twisted again before soaking in 10% potassium alum solution (60°C) for 2 min. The clothes were cleaned and damped repeatedly. To finish the dyeing process, the sample was soaked in 10% NaCl solution (60°C) for 2 min, followed by washing and drying.

Color analysis

An UltraScan VIS Spectrophotometer (HunterLab, USA) was used to obtain average values for L*a*b*. Fabric sample colors were generated by the software automatically.

Statistical analysis

The obtained data were analyzed using Microsoft Excel 2016 for Windows. The data were analyzed by one-way ANOVA and Duncan's mean comparison test at the 5% significance level (Steel et al., 1997).

RESULTS AND DISCUSSION

It was found that the 10% (w/v) of *Amaranthus viridis* (AV-), *Carissa carandas* Linn. (CC-), and *Tamarindus indica* (TI-), extracts revealed the color of pale yellow, light pink, and light brown, respectively as indicated in Figure 1.

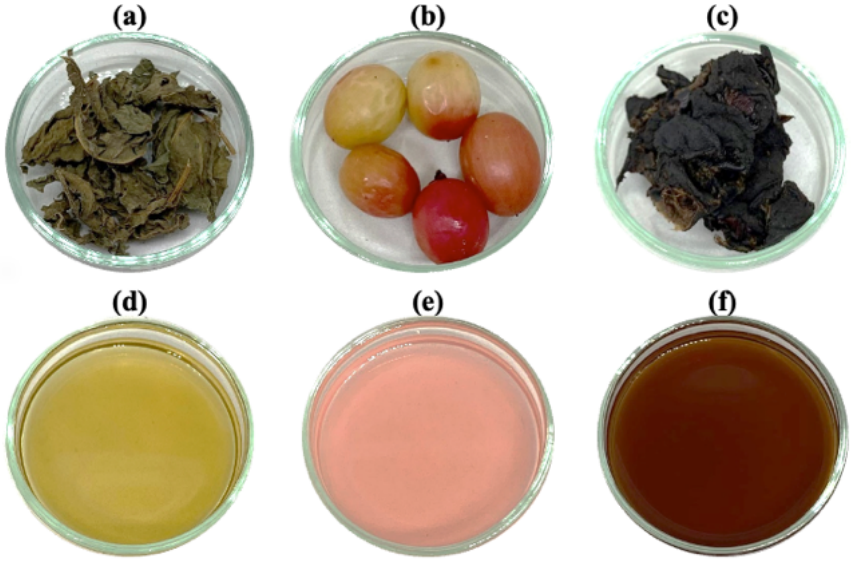

Figure 1. The appearance of (a) *A. viridis*, (b) *C. carandas*, (c) *T. indica* and the extracts of (d) AV-extracts, (e) CC- extracts, and (f) TI- extracts.

The color of the AV- extracts was pale yellow, as documented by Koyyati et al. (2014). It might consist of plant pigments such as carotenoids and soluble flavonoid derivatives, which were responsible for their yellow. In contrast, CC- extract was exhibited in light pink due to its composition of anthocyanin, as reported by Sarkar et al. (2018). However, the TI-extract was elicited in light brown, which resulted from enzymatic browning and Maillard reaction, as indicated by Obulesu and Bhattacharya (2011). A phytochemical was conducted to investigate these extracts' properties, and the results are in Table 1.

Table 1. Phytochemical properties of *A. viridis*, *C. carandas*, and *T. Indica* extracts.

Extract	pH	IC_{50} of DPPH (mg/mL)	FRAP (µg GAE/mg DW)	TPC (µg GAE/mg DW)	FC (µg QE/mg DW)	Tannin (µg ECGC/mg DW)
A. viridis	7.0±0.04^{c}	0	7.48±0.46^{b}	0.16±0.00^{c}	0.21±0.02^{c}	1.38±0.14^{b}
C. carandas	2.6±0.00^{b}	35.04±3.55	47.42±2.22^{a}	0.93±0.03^{b}	0.92±0.15^{a}	7.55±0.41^{a}
T. indica	2.2±0.01^{a}	163.07±9.74	36.63±4.68^{a}	1.03±0.04^{a}	0.66±0.01^{b}	6.85±0.03^{a}

^{abc}Within a column, means without a common superscript differ (P<0.05).

The crude extract prepared by dissolving in hot DI water showed the pH value of CC-extract was close to the control sample TI-extract. It might result from the high composition of organic acids of CC-extract, such as ursolic acid (Neimkhum et al., 2021; Bhosale et al., 2020). However, the AV- extract also consisted of organic acids such as oxalic acid (Kheyrodin, 2009) and many alkaloids (Sasikumar et al.,2015), which contributed to the increase of the extract pH. Unfortunately, the reducing activity IC_{50} of AV- extract possessed very low measured by DPPH assay, whilst the CC- extract showed more excellent reducing activity than control sample TI-extract. This phenomenon was insisted with the ferric ion reduction, an indicator of the electron donor, characteristic of the antioxidant action of the polyphenols (Khiya et al.,2021) measured by FRAP assay. However, to investigate the source molecules of reducing power, the total phenolic content (TPC) assay was conducted using Folin– Ciocalteu colorimetric method. The highest TPC of plant aqueous extracts was found in TI- extract following CC- extract and AV- extract. This indicates CC-extract had a high ability to reduce Folin–Ciocalteu reagent nearly to the control sample. In addition, CC-extract also exhibited the highest flavonoid tannin content, contributing to the reducing properties. Therefore, CC-extract might be an excellent tentative plant for using traditional cotton fabrics dyeing with *S. cusia* instead of TI- extract. To characterize the composition of the extracts, the UV-Vis spectra of the extract were recorded by spectrophotometry and shown in Figure 2.

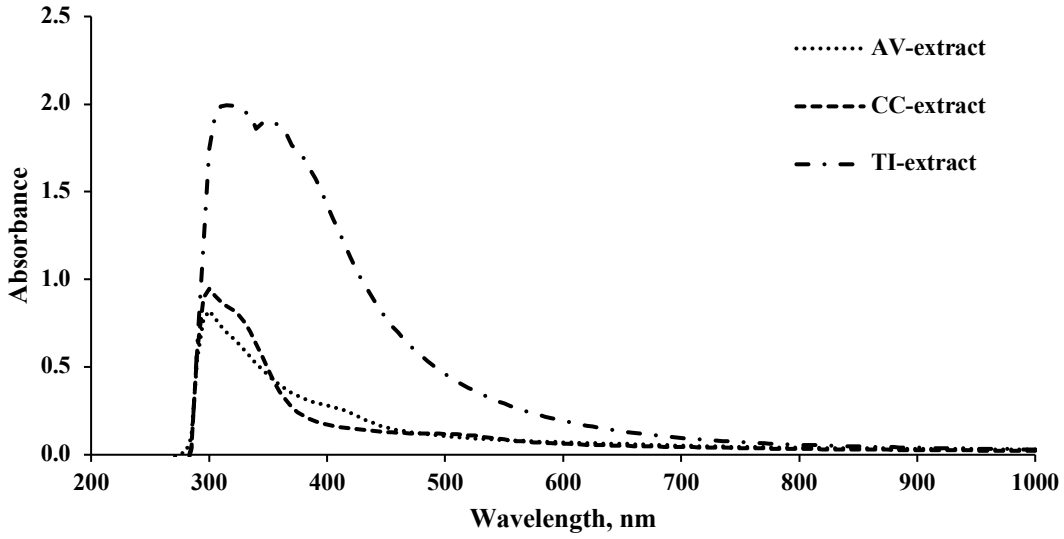

Figure 2. UV-VIS spectra of 10g/L crude extracts in distilled water.

As indicated in Figure 2, it was found that the characteristic spectra showed absorptions of AV-extract in the 290 nm to 360 nm, CC-extract in the 290 nm to 380 nm, and TI-extract in the 305 nm to 555 nm. The absorption range of 300-400 nm might be considered to originate from the $\pi \rightarrow \pi^*$ transitions in the B ring for the cinnamoyl system of flavonoids in the extract, as indicated by Mongkholrattanasit et al. (2011). However, to investigate the reducing properties, the extracts were employed in fabric dyeing, and the results are shown in Figure 3.

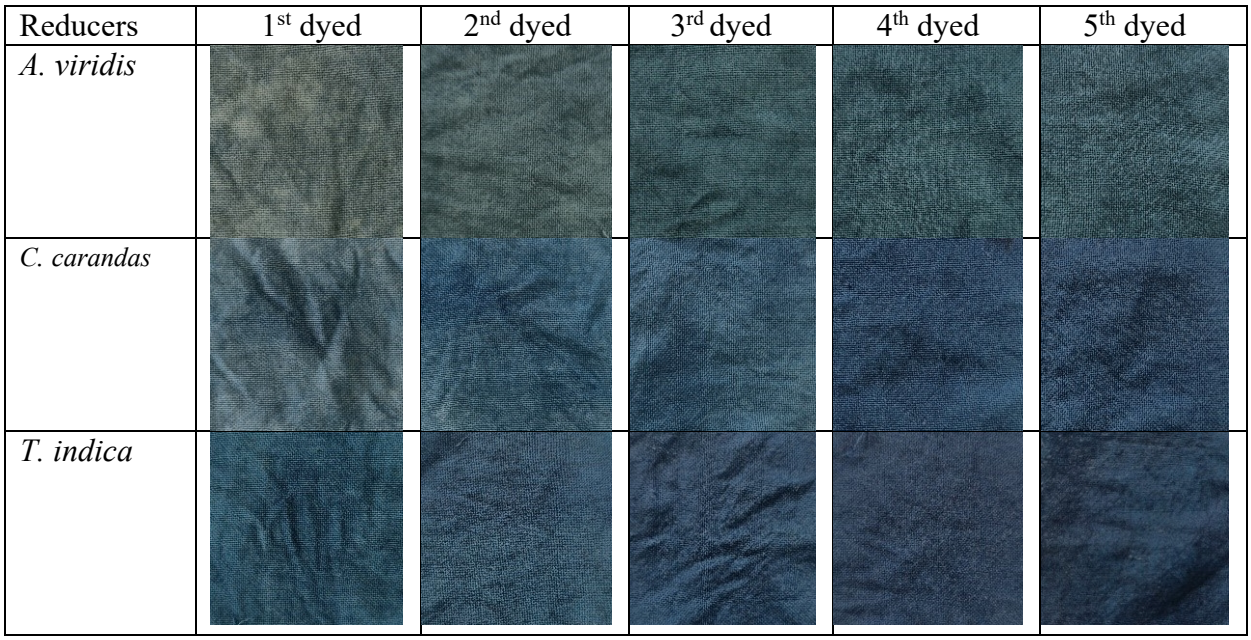

Figure 3. The color was obtained from dyed fabrics with *S. cusia* using *A. viridis*, *C. carandas*, and *T. indica* extracts as reducers.

The color appearance of using each reducing agent is presented in Figure 3. Fabric dyed with *S. cusia* and AV-, CC-, or TI-extracts showed light blue in 1st dyeing and increasing intensity in the following dyeing. The 5th dyed cotton fabric with *S. cusia* and AV-extract possessed pale blue, whereas the cotton fabric with *S. cusia* and CC-extract showed dark blue using control TI- extract. However, the proper mordant was applied as a dye fixative. In this experiment, the 10% (w/v) bio-mordant tea solution was used, and the color obtained was presented in Figure 4.

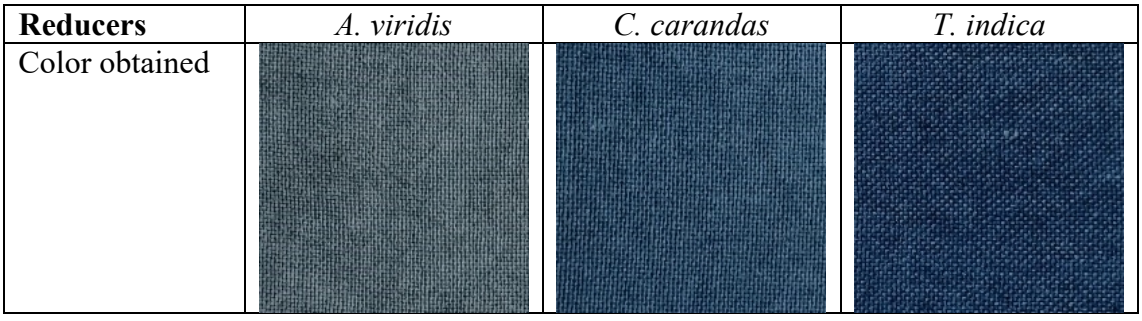

Figure 4. The appearance of dyed fabrics after 5th dyeing, mordanting with tea, and ironing.

It is clearly indicated that 10% (w/v) of CC-extract showed the blue color shade of the fabrics dyed, which is visually similar to TI- extract. Therefore, the dyed fabrics were colorimetrically observed, and the data were presented in Table 2. From Table 2, It is clear that the color shade fabrics dyed with 10% (w/v) of CC - extract were similar to that using 10% (w/v) of TI-extract but not 10% (w/v) of the AV-extract. Therefore, it can be concluded that the CC-extract had a high potential of reducing agent for *S. cusia* dyeing. To optimize the concentration of CC-extract in dyeing, the various concentrations of CC-extract were used before mordanting. The results are presented in Figure 5.

Table 2. The color values of 5th fabrics dyed with *S. Cusia* using AV-, CC-, and TI-extracts as reducers.

Reducers	L*	a*	b*
AV-extract	57.86±1.73[b]	-4.80±0.09[b]	-5.99±0.74[b]
CC-extract	39.27±0.47[a]	-3.26±0.13[a]	-17.26±0.58[a]
TI-extract	36.09±1.74[a]	-3.78±0.09[a]	-17.95±0.75[a]

[abc]Within a column, means without a common superscript differ (P<0.05).

Table 3. The color values of 5th fabrics dyed with various concentrations of CC-extract as reducers.

Con.	L*	a*	b*
15% dyed	40.95±2.75[a]	-3.91±0.25[a]	-15.54±1.59[a]
20% dyed	33.35±0.52[b]	-2.31±0.24[b]	-18.44±0.33[a,b]
25% dyed	29.97±0.81[c]	-1.37±0.30[c]	-19.30±0.21[a,b]
30% dyed	29.27±0.47[c]	-1.16±0.24[c]	-18.87±0.22[a,b]
35% dyed	28.27±0.54[c]	-0.89±0.19[c]	-19.27±0.54[b]

[abc]Within a column, means without a common superscript differ (P<0.05).

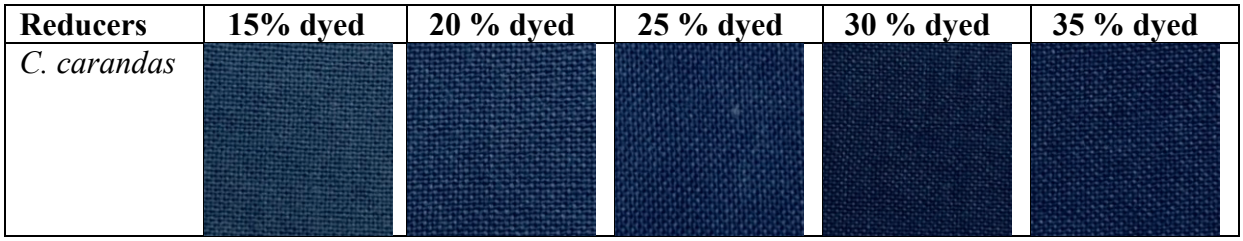

Figure 5. The **color** was obtained from dyed fabrics with *S. cusia* using various concentrations of CC extracts as reducers.

From Figure 5, It was found that the blue color shade of the fabrics dyed with various concentrations of CC-extract visually observed was intensified with a higher concentration. The data from the colorimetric analysis are shown in Table 3.

It was found that fabrics dyed with *S. cusia* and CC-extract showed a higher color strength and responded to a higher concentration of CC-extract. The optimal concentration for using equivalence to 10% (w/v) of TI-extract was between 15% and 20% (w/v) of CC-extract.

CONCLUSIONS

In summary, *C. carandas* extracted with hot water is suitable for use in the *S. cusia* dyeing process and *T. indica* extract. The 15-20% (w/v) of CC-extract possessed excellent reducing properties for blue color formation on cotton fabric. Therefore, CC-extract has a high potential reducing agent in traditional cotton fabric dyeing. This presents a promising low-cost reducing agent for green dyeing fabric in the near future.

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Case study: Evaluation of implementations of good dairy farming practice (GDFP) at SRC Animal Health Dairy Farm, Nakhon Ratchasima, Thailand

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ABSTRACT

The objective of this case study was to implement dairy farm practices in Thailand using Good Dairy Farming Practices (GDFP) as guideline tools. This observation was carried out at SRC Animal Health Dairy Farm in Pakchong, Nakhon Ratchasima, Thailand, for one month, from October 20 until November 20, 2019. The implementation process was conducted by four mimic inspectors from the program of Animal Science, Faculty of Animal Science, Universitas Brawijaya, Indonesia. The GDFP tool was developed by the Food and Agriculture Organization of the United Nations (FAO) and the International Dairy Federation (IDF) since the year 2011. The implementation covered seven areas of the farm operation, including animal health, milking hygiene, nutrition, breeding and reproduction program, animal welfare, environment, and socio-economic management. Summary of implement activities included meeting with the farm manager and staff, observing farm activities, inspecting farm facilities, inspecting farm documents on the checklist, and interview. Data indicated that the type of cow raised at SRC Animal Heath Farm was the crossbred Friesian Holstein. The overall result of the implementation indicated that the mean technical score was 3.73 on 4 point scale. The score reflected that farm management was in a good category. In addition, some quality perspectives should be required to adopt or apply in order to gain better scores and international recognition based on the recommendation tools of FAO and IDF standards.

Keywords: dairy cattle, good dairy farming practices, milking, management

INTRODUCTION

Dairy cattle are an animal initiated specifically to produce milk because of their ability to produce abundantly. Friesian Holstein (FH) is a breed of dairy cattle that is widely kept in Indonesia because of its high milk production. According to Prastowo *et al*. (2019) this breed carries the superior genetic potential for milk production by being able to produce 5,217 kg of milk per year. Also, FH is able to adapt to tropical and sub-tropical countries under temperatures range 5-25°C.

During the late 1950's Thailand progressively imported dairy items. Over the past 68 a long time the commonplace little holder has extended. Concurring to Suryasathaporn *et al*. (2012) Thailand has the speediest developing dairy industry in tropical Asia, with 610% more drain generation in 2006 than within the period 1990/92; a development rate of 38% per annum. On the other side moreover note, that the industry has too made a few expansive commercial ranches. A great illustration of this unused drift is "SRC Creature Wellbeing Farm" in Pakchong, Nakhon Ratchasima, Thailand. The cultivation and handling plant has been working since 2017; the cultivation managing of 207 cattle with milk production per day of 2,103.4 kg and a normal generation of 18.45 kg/cow/day. The cultivate and its offices can be considered one of the most excellent dairy operations in Thailand. Agreeing with Quddus (2012) to extend the country's drain yield, a reasonable methodology centering on tall yielding breeds and progressed administration innovation ought to be embraced for impressive dairy improvement. A few of the extraordinary highlights incorporate: (i) bolstering castles an adjusted proportion were at slightest 50% of supplements determine from roughage; (ii) a target generation not more than 2,500 kg of drain guaranteeing the lactations cattle sound through every day controlling schedule; (iii) a bolster supply that's secured primarily through contract developing operations by adjacent smallholders; and (iv) fluid excrement prepared into crops fertilizer and dispersed to crops developing operations by adjacent smallholders.

The strategy of support of dairy bovines ought to be considered persistently, all together, and proficiently both from the perspectives of breeding and generation, bolster and drinking water,

administration walled in area and gear, wellbeing, and creature welfare. Concurring to Asminaya *et al*. (2020), the application of GDFP for dairy cultivation influences the generation of drain created by animals. A higher level of drain castle upkeep administration, within the sense of great support, can be guaranteed drain production in dairy animals is additionally great. The objective of this study was to implement dairy farm management in Thailand based on Good Dairy Farming Practices (GDFP), including animal health, milk hygiene, feeding and nutrition management, breeding and reproductive management, animal welfare, environment, and socio-economic management.

MATERIALS AND METHODS

Location and time

The implementation was conducted in Pakchong, Nakhon Ratchasima, 30130, Thailand from 20th October to 20th November 2019. The altitude of Pak Chong district is 331 masl (meters above sea level); the latitude is 14°58:50" N 102°6:00" E. The average temperature was 22-29°C, and the humidity was around 62-88%.

Methods of implementation

The implementation was conducted using four undergraduate students from the program of Animal Science, Faculty of Animal Science, Universitas Brawijaya, Indonesia, as mimic inspectors. Summary of implement methods included meeting with the farm manager and staff, observing farm activities, inspecting farm facilities, inspecting farm documents on the checklist, and interviews. The implementation covered seven areas of the farm operation, including animal health, milking hygiene, nutrition, breeding and reproduction program, animal welfare, environment, and socio-economic management based on the guideline's implementation of GDFP (Good Dairy Farming Practice) according to FAO and IDF (2011). The evaluation quality was based on four quality scales (Table 1).

Statistical analysis

Data from each panel were combined and analyzed using descriptive statistics (Steel et al., 1997).

Table 1. Farm performance conversion score.

Average Score of GDFP	Quality Score	Category
0.00-0.50	0	Very Bad
0.51-1.00	1	Bad
1.01-2.00	2	Poor
2.01-3.00	3	Enough
3.01-4.00	4	Good

RESULTS AND DISCUSSION

The success of a dairy farming business can be seen from the level of knowledge and skills as well as the application of the management and technical aspects of raising livestock owned by the farm. The standard for assessing the success of a dairy farming business according to FAO and IDF consists of several aspects, including animal health, milk hygiene, nutrition (feed and water), breeding and reproductive, animal welfare, environment, and socio-economic. The results of the evaluation of the success of smallholder dairy farming based on the score of the application of GDFP at SRC Farm can be seen in Table 2. The score of the technical aspect of maintaining private farm dairy cattle at SRC Farm based on the GDFP standard was an average of 3.73, which is in a good category (Table 2).

Table 2. The overall mean of implement score.

No	Good Dairy Farming Practice Aspects	GDFP Score	Category
1	Animal health	3.71	Good
2	Milking hygiene	4.00	Good
3	Nutrition (feed and water)	3.42	Good
4	Breeding and reproductive program	4.00	Good
5	Animal welfare	3.77	Good
6	Environment	3.80	Good
7	Socio-economic management	3.40	Good
	Average	**3.73**	**Good**

The data showed that most of the farm management is slightly weak in some technical aspects such as nutrition and socio-economic management which scores were 3.42 and 3.40, respectively. The application of technical aspects of good maintenance on dairy farms certainly affects the productivity of dairy farms. Suherman (2005) stated that the success of a dairy farming business and increasing milk production depends on the application of good maintenance, feeding, reproduction, genetic, and environmental

management. Although it was in the rainy season, milk hygiene and breeding and reproductive programs were scores of 4.00 which are in the good category. In addition, aspects of environmental management, animal welfare and animal health received scores of 3.80, 3.77, and 3.71 respectively, which are also in the good category.

Animal health

Animal health is crucial when running a farm by keeping an eye on animal health and preventing animal disease. Moreover, it can be crucially affected both economy and the safety of the food supply. The production of healthy livestock will ensure a safe and hygienic; it will also keep consumer prices stable. The GDFP score in animal health was 3.71 which is in the good category reflecting well-taking care of dairy cows (Table 3). The lowest score (2) came from the vermin control program; even though the treatment was flawless, the prevention was not quite enough. The vermin need to be exterminated to prevent unwanted diseases; vermin or pests are usually harmful to dairy cows, directly and indirectly. The farm has already programmed the routine vaccination for Foot-and-Mouth Disease (FMD) and plume disease for the calf, heifers, and cows. In addition, the farm also provides foot and mouth disease vaccination every four months; also, prevention and treatment for several diseases are extraordinary. For example; first, mastitis, detection using the Californian Mastitis Test (CMT), is detected when the milk forms a thick gel caused by alkyl aryl sulfonate and is treated with antibiotics injection to the mammary gland. The most commonly treated for clinical mastitis is intramammary administration of antibiotics to the mammary gland cavity (Kromker and Leimbach, 2017). Second, Laminitis, Laminitis occurs when the dermal layers inside cow claws become inflamed (Burger, 2017). Also known as foot rot, the treatment gives hoof trimming every two days. The recovery period is approximately four until six months. Third, diarrhea, calf diarrhea is most known for its damage to the farm, it can cause serious financial and animal welfare implications, not just in dairy but also in beef cattle as well (Muktar et al., 2015). For prevention, the calf is injected with the vaccine to increase body immunity, and if the calf still gets diarrhea, it will be continued with the injection of medicine. Lastly, metritis, the treatment is an injection of metricure and antibiotic, The cows that suffer from metritis require a systemic antibiotic treatment because of severe illness and risk for death but for assessing treatment success is still inconsistent (LeBlanc, 2008), for the complication, as the difficulty of giving birth, it is major to perform Cesarean section to relieve the pain of the cow and prevent the cow from death.

Milk hygiene

Dairy cow hygiene is used as an indicator of animal welfare because it provides information about the quality of life of animals and the quality of livestock facilities (Hultgren and Bergsten, 2001; Blokhuis, 2009; Sant'Anna and Costa, 2011). SRC farm had a GDFP value of 4 (Table 4), which means that the milk hygiene aspect has a good value and quality reflecting its specializes in managing milk hygiene. The milking process at SRC Farm carried out twice a day, in the morning at 5.30 and in the afternoon at 4.30. This is in accordance with what is conveyed by FAO and IDF explaining the milking process that can be done twice a day at 7 am and 5 pm. Before milking, cows were doused with water before entering the milking room to lower their body temperature, refreshing the cows so they can avoid stress. There is ample evidence showing that air can effectively reduce stress indicators such as respiratory rate and body temperature (Gaughan, et al., 2004; Kendall, et al., 2007; Legrand, et al., 2011). SRC Farm has a Standard Operating Procedure (SOP) for milking to produce milk of good quality and safe for consumption. SOP is one of the important aspects that will affect the production of livestock and in terms to table 4, the existence of SOPs and their observance gives a good value to SRC Farm. The SOP includes cleaning the nipples with a warm towel to reduce the number of bacteria and dirt. Disinfection of dairy cows before milking has been shown to reduce the number of bacteria on the nipple skin (Gleeson et al., 2018). Dip the nipple into the iodine solution and then wipe it again with a new warm towel until the nipple is clean and dry. The milking process can be started by making sure the nipples are clean and dry (FAO and IDF, 2011). Milking begins with manual flushing 2-3 times and is discarded to remove bacteria, then a CMT test is performed to detect mastitis. CMT is used to diagnose subclinical mastitis in milk-producing cows (Tolosa, et al., 2013); then proceed to the milking process with a machine for about 10-15 minutes per head. After the milking process is complete, each nipple is immersed in the iodine solution without being washed until the next milking. The finished cow will return to the pen and pass through the disinfectant pool next to the milking room.

Table 3. The score of implementation on the animal health aspect.

Good dairy farming practice	Defining Factors	GDFP Score	Category
Managing the herd to prevent disease	The breeds and animals are suited to the environment and farm management	3	Enough
	Managing the farm to fit with the herd size and the availability such as land, infrastructure, feed, and others	4	Good
	Vaccinated animals as a requirement by local animal health authorities	4	Good
Prevention of any disease in the farm	Buy animals that are up to standard such as health status and quarantine after deliverance	3	Enough
	Animal transport and the farm is free of disease	4	Good
	Aware of the risk of adjacent land and boundaries of neighbors	4	Good
	Limit access of people and wildlife in the farm	4	Good
	Have a pest control program in the farm	2	Poor
	Only use medicine or treatment that are according to expiration date used	4	Good
Hold an effective management program for the herd	Have an identification system that allowed to record all the animals from birth to death	4	Good
	Create an effective health management program for herd that are focused on prevention of disease to the standard of regional or national requirements	3	Enough
	Check animals daily for disease sign	4	Good
	Sick animals must be treated quickly and appropriately	4	Good
	Sick animals must be isolated	4	Good
	Separate milk from sick animals and animals under treatment	4	Good
	Keep the records for all the medication and the identification of animals that are being treated	4	Good
	Prevention of animals that can cause public health (zoonosis)	4	Good
Traceability feed stuff that are brought to the farm	Use the chemicals that are approved and used under regulation	4	Good
	Use the chemicals by following the directions and the right dosage, also observe the expiration date	3	Enough
	Only used medication by veterinary and formulated by veterinarians	4	Good
	Store the medicine and veterinary medicine safely and disposed it appropriately	4	Good
	Average	**3.71**	**Good**

One dairy cow at SRC Farm is capable of producing 7-15 kg of milk daily which will be stored in the cooling tank. The cooling tank used to store milk is between 3 to 4 °C. This is in accordance with FAO and IDF that milked milk can be stored in a cooling tank with a temperature of 3 to 4ºC. The milk will be transported to the processing center at 2 p.m. After the milking process is complete for all cows, the milking room is cleaned by manual and automatic systems. One of the things that need to be considered in the milking room of this farm is the presence of biosecurity at the entrance of the milking room. There is a disinfectant liquid to prevent bacteria from being carried by workers into the milking room. To have a good dairy farming system, we must ensure that the housing environment is always clean (FAO and IDF, 2011). Cleanliness and good facilities give the GDFP value in terms of 4 are classified as good.

Nutrition (feed and water)

The production of ruminants is largely determined by the availability of feed and drinking water. Types of feed given to dairy cows, namely forage and concentrate. Adequacy of basic nutrients in dairy cattle is used for growth, reproduction, lactation, and locomotion. Within the perspective of bolster and drinking water administration, it can be seen that the GDFP score got a normal score of 3.42. It can be said that the execution of nourishment administration at SRC Cultivate is within the good category (Table 5). The least GDFP score within the destitute category appears in Guarantee the dietary needs of creatures are met (2); Guarantee the nourish encouraged to dairy creatures is fit for a reason and will not adversely affect the quality or security of their drain or meat, where conceivable, source creature bolster from providers having an endorsed quality affirmation modified input and keep records of all bolster or nourish fixings gotten on the cultivating (3). Ensuring the nutritional needs of

animals are met is not good enough because the farm uses a total mixed ration (TMR) which the TMR formulation may not update. The TMR formulation should be evaluated and updated every two years so that the nutritional needs of dairy cows are in line with milk production. Heifer cattle are fed rice straw which is classified as poor-quality forage. Rice straw is the waste product from the agricultural product that contains 2% of crude protein. The low forage quantity available in the dry season and low-quality protein concentrated feed leads to a decrease in the productivity of dairy cattle (Susanti and Marhaeniyanto, 2007). The sub-aspect of how to feed is included in the good category because the feed is given after the milking process is complete, this aims to reduce contamination in milk.

Table 4. The score of implementation on the aspect of milk hygiene.

Good dairy farming practice	Defining Factors	GDFP Score	Category
Ensure milk is treated properly after milking	Ensure milk is refrigerated or sent for processing within the specified time	4	Good
	Ensure the milk storage area is clean and tidy	4	Good
	Ensuring equipment for milk storage is sufficient to hold milk at a predetermined temperature	4	Good
	Ensure equipment for milk storage is cleaned and sanitized regularly or after each milk collection	4	Good
	Ensure unhindered access to mass milk collection	4	Good
Ensure that milking activities are carried out in hygienic conditions	Make sure the cage environment is clean at all times	4	Good
	Make sure the milking area is always clean	4	Good
	Ensure sufficient water supply, good water quality and regularly maintained	4	Good
	Making sure the milker always follows basic hygiene rules	4	Good
	Ensure equipment for milking is cleaned regularly disinfected or after each milking	4	Good
Ensure that milking activities do not injure animals or introduce contaminants into milk	Identify each individual animal that requires special milking management	4	Good
	Ensure that the udder is properly prepared for milking	4	Good
	Milk regularly using a consistent milking technique	4	Good
	Separating harvested milk from sick or treated animals for proper disposal	4	Good
	Ensure equipment for milking is properly maintained and installed	4	Good
Average		**4**	**Good**

Table 5. The score of implementing SRC farm's good dairy farming practice in aspects of nutrition

Good dairy farming practice	Defining Factors	GDFP Score	Category
Secure feed and water supplies from sustainable sources	Arrange ahead to guarantee that the herd's bolster and water requisites are met	4	Good
	Execute economical supplement, water system and bug administration hones when developing nourish	4	Good
	Source cultivate inputs from providers actualizing economical frameworks	4	Good
Ensure animal feed and water are of suitable quantity and quality	Guarantee the dietary needs of creatures are met	2	Poor
	Guarantee the bolster bolstered to dairy creatures is fit for reason and will not adversely affect the quality or security of their drain or meat	3	Enough
	Guarantee appropriate quality water is given and the supply is routinely checked and kept up	4	Good
	Utilize diverse gear for taking care of chemicals and bolster stuffs	4	Good
	Guarantee chemicals are utilized fittingly on pastures and scrounge crops and watch withholding periods	4	Good
	As it were utilized affirmed chemicals for treatment of creature bolsters or components of creature bolsters and watch withholding periods	4	Good
Control storage conditions of feed	Isolated nourishes planning for distinctive species	4	Good
	Guarantee suitable capacity conditions to dodge bolster deterioration or defilement	4	Good
	Declines mildew covered or sub-standard bolster	4	Good
Ensure the traceability of feedstuffs brought on to the farm	Where conceivable, source creature nourishes from providers having an affirmed quality confirmation modified input	3	Enough
	Keep records of all bolster or bolster fixings gotten on the cultivating	3	Enough
Average		**3.42**	**Good**

Feeding management at SRC farms is differentiated based on the growth phase and ad libitum feeding system. Feeding forage for lactating cows and dry cattle is carried out twice a day in the morning and evening in a fresh state while feeding for heifers is carried out 3 times a day in the morning, afternoon, and evening. Meanwhile, the administration of concentrate for the heifer is carried out in the morning and evening only. Feeding must be in an appropriate percentage between forage and concentrate. The highest GDFP score (4) with the great category appeared in Secure bolster and water supplies from economical sources and Control capacity conditions of the bolster. The SRC farm cultivates and guarantees the accessibility of nourishment for all animals by conducting a coordinated cultivating framework in which SRC cultivate has its claim crops where the plants are utilized in making silage. Maize silage is the most bolster in SRC Dairy Cultivate. This feed silage storage will last as long as three (3) months. To avoid unsustainable water use or water deficiency, the dairy industry must focus on the development of drought-tolerant crops (Heisey and Rubenstein, 2015); shifting to water-efficient and drought-tolerant crops (Marshall et al., 2015); using soil-management practices that increase water retention, such as conservation tillage, crop residue management, or cover crops (Marshall et al., 2015). The storage system of feed materials at the farm uses FIFO (First in First Out). This means that the new feed material coming stored and feed ingredients that have been provided are used to feed material have been used up. Storage of feed materials using storage methods in the packaging (sacks) and for storage of feed ingredients and medicines/chemicals are stored in separate warehouses.

Breeding and reproductive program

In dairy cows, reproductive management and breeding have a very crucial influence on the economic aspect. Therefore, it is necessary to manage the breeding and reproduction of the right cows so that the productivity of dairy cattle runs smoothly. According to Ribeiro et al. (2012) For each cultivate and for each bovine, there's an ideal time for pregnancy, which is for the most part affected by the level of generation, tirelessness of lactation, and equality. dairy cattle business because it will affect the success of the livestock business. The score of GDFP for the breeding and reproductive program at SRC Farm is in a good category (4) as shown in Table 6. Based on observations in the field, it is known that the breed of dairy cattle kept on the farm under study is Frisian Holstein (FH) which belongs to the breed of cows with high milk production with characteristics including dominant coat color of black and white stripes and in small quantities, there is a with red and white stripes, the head is relatively long, wide and straight with relatively short horns that point laterally and curve forward, and have a calm and docile temperament (Efata, 2018).

Breeders at the research site are breeding by crossbreeding between FH cattle and Jersey cattle. It aims to produce high-quality and quantity milk production. As we know, FH breed produces a high quantity of milk, while Jersey breed produces high quality (high-fat content) of milk. According to Makin (2011), the milk-producing castle FH is the highest in the world with an average of 6000 liters per lactation and a fat percentage average of 3.5%, which varied from 2.5 to 4.3%. Choose livestock seeds that have entered the age of ready to mate. The conception method aspect is considered good because this farm already uses breeding technology such as synchronization estrus, artificial insemination, and embryo transfer. The condition of the knowledge aspect of lust is in a good category because in this farm a recording system related to the reproductive cycle has been recorded on the computer and the presence of an IoT necklace sensor makes it easier for veterinarians to monitor the movement of cows that are about to enter estrus. Ideal development rates of yearlings are basic for future creature execution. In arrange to maximize lifetime generation, Holstein yearlings ought to calve at around 23 to 24 months of age with ~85% of grown-up body weight (Gabler et al., 2000; Etema and Santos, 2004). Yearlings calving at a more youthful age (i.e., 25 months) have no changes in efficiency at, to begin with, lactation (Gabler et al., 2000; Etema and Santos, 2004), with extra days of useless life and nourishment costs. This appears that smallholder dairy ranchers at SRC cultivate are exceptionally concerned about the administration of breeding and generation viewpoints.

Table 6. The score of implementing SRC farm's good dairy farming practice on breeding and reproductive aspects

Defining Factors	GDFP Score	Category
The breed of cattle	4	Good
Selection method	4	Good
Conception method	4	Good
Knowledge about estrus	4	Good
Age of first calving	4	Good
Mating after first calving	4	Good
Calving interval	4	Good
Average	**4**	**Good**

Animal welfare

The welfare of an animal depends on how it perceives the situation in which it lives (Keeling, 2013). There are five principles of animal welfare;

the animal never feels hungry, thirsty, and malnourished; the animal does not feel physical and thermal discomfort; the animal is free from injury, disease, and pain; the animal is mostly able to express its normal pattern of behavior; animals do not feel fear and distress. Based on the data from our research, SRC Animal Health has implemented Good Dairy Farming Practices in the Animal Welfare aspect. The results show the number 3.77 which is in the good category. The score of implementing SRC Farm's Good Dairy Farming Practice on the Animal Welfare aspect can be seen in Table 7.

In SRC Animal Health Farm, the animal fed very well and properly (Table 5). The farm applies an integrated farming system that consists of not only a dairy cattle farm but also a wide area of plantations such as grasses, fruits, and vegetable gardens. It makes the feed availability so abundant because the farmer makes their own silage from their own land. This farm also implements very good health management by routinely providing vaccines, disinfectants, vitamins, and adequate nutrition, and is provided by a trained veterinarian and nutritionist. SRC Farm applies free stall cages to all parts of the farm, including dairy cattle, breeding cattle, heifer, and dry cattle. Comfort in livestock is one of the important factors that influence the profitability of dairy farming because of its relationship with their productivity.

However, SRC Animal Health has some activities and routines that are not in accordance with animal welfare principles. Most of the workers on the farm handling the cattle or moving the cattle from one cage to another use improper handling and restraint techniques that could make the cattle to feel stress, fear and distress due to insufficient training in handling and restraint or awareness of animal welfare principles. Besides, the relationship between mother and baby after birth is not supporting the freedom to express normal behavior. The newborn baby calf will be separated from the mother to a pen for the calf right after the mother gives birth to prevent the mother-calf bonding. The baby will be given colostrum for a few days and given formula milk instead. This is done for perceived benefits from an economic point of view, by accumulating higher milk yields for human consumption (CIWF, 2013)

Table 7. The score of implementing SRC farm's good dairy farming practice on the animal welfare aspect

Good dairy farming practice	Defining Factors	GDFP Score	Category
Ensure animals are free from thirst, hunger and malnutrition	Provide adequate feed and water to all the livestock every day	4	Good
	Adjust stocking rates and/or supplementary feeding to ensure sufficient supply of water, feed and fodder supply	4	Good
	Protect animals from toxic or harmful plants and other hazardous substances	4	Good
	Provide a good quality water supply which is regularly checked and maintained	4	Good
Ensure livestock are free from discomfort	Design and construct buildings and handling facilities to be free of obstructions and dangers	3	Enough
	Provide enough space and clean bedding	4	Good
	Protect animals from bad weather conditions and their consequences	4	Good
	Provide animal housing with adequate ventilation	4	Good
	Provide suitable footing and flooring for housing and animal traffic areas	3	Enough
	Protect animals from injury and distress during loading and unloading also provide suitable conditions for transportation	4	Good
Ensure livestock are free from injury, disease and pain	Have a program of regular checkups and effective herd health management	4	Good
	Do not use practices and procedures that cause unnecessary pain	4	Good
	Follow appropriate weaning and birthing practices	4	Good
	Have proper procedures for marketing young dairy cattle	4	Good
	Protect against lameness	4	Good
	Milk the lactating dairy cattle regularly	4	Good
	Avoid bad milking practices as it can injure dairy cattle	4	Good
	Avoid unnecessary stress or pain when animals have to be euthanized on the farm	4	Good
Ensure livestock are free from fear	Consider herd management routines and dairy cattle behavior when developing livestock infrastructure	4	Good
	Provide competent husbandry skills, stock handling and appropriate training	2	Poor
	Use appropriate facilities and equipment for stock handling	3	Enough
Ensure the dairy cattle can engage in relatively normal patterns of livestock behavior	Ensure the dairy cattle can engage in relatively normal patterns of livestock behavior	4	Good
	Average	**3.77**	**Good**

Environment

SRC Farm is located in the Amphoe Pak Chong district, Nakhon Ratchasima Province, Thailand. Pak Chong District Altitude is 331 masl. The temperature in this place ranges from 22 to 29°C with humidity around 62 to 88%. Cattle are affected at any time when the ambient temperature is more than their thermoneutral zone, which ranges from 25°C (Atrian and Shahryar, 2012). The farm has a total area of 48 ha which is divided into 4 sections including housing, buildings (offices, laboratories, workers' houses, milking facilities, and warehouses), waste lagoons, and crop fields. The cage area has an area of 22 ha, a building area of 3.7 ha, a WWTP area of 0.3 ha, and a planting area of 22 ha. SRC Farm is located in an area with a sufficient water supply and abundant feed availability. Livestock can meet their nutritional needs properly. The drainage owned by SRC Farm is also quite good so that the cage is clean and not damp. Spatial conditions and livestock facilities are quite good indicating that the milk production produced is also good. Good layout and environmental conditions give SRC Farm a GDFP value which is seen in Table 8 is good.

The SRC farm cleans the garden two to three times daily manually by workers and using a tractor. The manure produced by livestock is cleaned and managed properly by the farm. The manure will be processed into fertilizer which will then be sold or used by SRC Farm itself. The rest of the waste in the form of water will be disposed of at the disposal of the SRC Farm so that it does not pollute the surrounding environment and this provides good value for environmental treatment in terms of GDFP. SRC Farm is still not able to manage the more complex biogas which will later be useful as an energy source other than electricity; so in table 8, there are still points that have a value of 3 or sufficient because they have not maximized the utilization of other resources and more complex processing. FAO and IDF (2011) explain that in order to have a good dairy farming system, we must implement proper practices to reduce, reuse or recycle livestock as waste.

Table 8. The score of implementing SRC farm's good dairy farming practice in environmental aspects

Good dairy farming practice	Defining Factors	GDFP Score	Category
Implementing an environmentally friendly farming system	Use agricultural inputs such as water and nutrients efficiently and sustainably	4	Good
	Minimizing the production of environmental pollutants from dairy farming	4	Good
	Managing dairy cattle to minimize negative impacts on the environment	3	Enough
	Choosing and using energy resources appropriately	3	Enough
	Maintaining and/or promoting biodiversity in agriculture	4	Good
Have an appropriate waste management system.	Implement appropriate practices to reduce, reuse or recycle agricultural waste	4	Good
	Manage waste storage and disposal to minimize impact on the environment	4	Good
Ensure that dairy farming practices do not have a negative impact on the local environment	Contains milk runoff on farms	4	Good
	Using chemicals, agricultural and veterinary fertilizers appropriately to avoid contamination of the local environment	4	Good
	Ensuring the overall appearance of the milking activity is appropriate for a facility where high quality food is harvested	4	Good
	Average	**3.8**	**Good**

Socio-economic management

The score of GDFP for socio-economic management at SRC is in a good category, with a score of 3.4 as shown in Table 9. However, the lower score came from the aspect of employing staff based on national laws and experience with the score of 2 being the poor category because some new crew members were inexperienced foreigners and needed to be trained before achieving professional performance in dairy practices. This is followed by implementing sustainable work practices, managing human resources effectively, ensuring that their working conditions comply with applicable laws and international conventions, inducting and working, and ensuring staff is appropriate for their work, given a score of 3 with a sufficient category. The busiest times for the workers are in the early morning and afternoon because their activities include the milking process, cleaning the stables, and feeding the cows. Because the farm activity had to run every day, the farm allowed one day of the week as a holiday depending on individual purposes. Research on leisure activities suggests that the activities people engage in during non-working time influence their level of Health and Well-being (De Bloom, 2011).

Table 9. The score in socio-economic management aspects

Good dairy farming practice	Defining Factors	GDFP Score	Category
Implement effective and responsible management of human resources	Implement sustainable work practices	3	Enough
	The staff on the farm is based on national law and experienced	2	Poor
	The farm is managed to maximize the human resource and made sure that the working conditions is in accordance to laws and international conventions	3	Enough
	Assure the farm to have a working environment that up to standard such as health and safety requirements	4	Good
Ensure farm tasks are carried out competently and safely	Have an equipment and procedure that are up to standard for dairy farming task	4	Good
	Able to train and educate the workers for their work	3	Enough
	Assure the staff to complete their task	3	Enough
	Pick the best people for the farm, whom are competent and able to communicate very well	4	Good
Manage the farm to secure their financial viability	Have a good financial management program	4	Good
	Using an agricultural practice that can reach productivity and profitability goals	4	Good
	Have a backup plan for a financial crisis	4	Good
Average		**3.4**	**Good**

While the highest aspect is ensuring the farm work environment complies with relevant occupational safety and health requirements, adopting farming practices that contribute to the company's productivity and/or profitability goals, having the appropriate equipment and procedures to perform dairy farming tasks, selecting competent people for training, planning ahead to manage financial risks, interventions and advice, and implement financial management systems get a score of 4 in the good category. This is because SRC Animal Health Dairy Farm has good financial management by implementing an integrated farming system. The main income from SRC Animal Health comes from exclusive milk production from dairy cattle and has relatively more expensive prices than milk in general. The additional income comes from processed cow waste that has been collected and processed into fertilizer, culling calf or cattle, plantation, and other agricultural products including watermelon, papaya, banana, and various other fruits and vegetables. Moreover, the worker makes their own silage from the plantation as cattle feed; so, it can reduce the feed cost which is the majority of production cost. SRC Farm adopts agricultural practices that contribute to the company's profitability and/or productivity goals, called Integrated Farming System. By implementing the integrated farming system, they produce their own food without having to buy products from outside which are relatively more expensive and increase production costs. Implementing an integrated farming system can generate additional income ranging from `9,000 to `200,000 per hectare, depending on the type of additional farming business, the amount involved and the effective combination thereof (Ponnusamy and Devi, 2017).

CONCLUSIONS

Good Dairy Farming Practice (GDFP) has a few viewpoints that must be followed to in carrying out the administration of dairy bovines such as creature wellbeing, breeding and regenerative program, drain cleanliness, bolstering and sustenance, creature welfare, environment, and socio-economic administration. According to the implementation outcome, SRC Animal Health Dairy Farm received an average technical score of 3.73 on 4 point scale that reflected that overall farm management is in a good category. Based on the tools of GDFP FAO and IDF standards, the farm has a great opportunity to expand its activity to an international level in terms of doing internships or research for international students around the world. In addition, some quality perspectives should be considered to adopt or apply on the farm to achieve full scores and international recognition.

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Effects of electrical conductivity and micro/nanobubbles in nutrient solutions of hydroponics on growth and yield of cherry tomato

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ABSTRACT

The objective of this study was to investigate the effects of electrical conductivity and micro/nanobubbles (MNBs) in nutrient solution culture under a hydroponic system on growth and yield of cherry tomatoes. The experiment was assigned using a 3x2 factorial arrangement in a completely randomized design. Factors were factor A, with three levels of electrical conductivity (EC) at 1, 2, and 3 mS/cm in the nutrient solution of the hydroponic system, and factor B, with two levels MNBs, including with or without the application of MNBs, respectively. The cherry tomato seedling was grown in a deep-flow technique (DFT) hydroponic system. The experiment was conducted in a greenhouse environment from July to October 2021 at the Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Lampang, Thailand. The results showed that the EC levels and MNBs did not increase plant height, inflorescence, leaf number per shoot, and leaf size. However, increasing EC from 1 to 2 mS/cm enhanced leaf greenness and chlorophyll fluorescence. On the other hand, the EC at 2 mS/cm with MNBs was the most effective, in particular on fruit size, fruit weight, and yield. Additionally, EC at 3 mS/cm with MNBs improved the total soluble solids of cherry tomato fruits. Results from this experiment suggest that levels of electrical conductivity and micro/nanobubbles have the potential to enhance growth, yields, and fruit quality of cherry tomatoes.

Keywords: tomato, electrical conductivity, micro/nanobubbles

INTRODUCTION

Tomato is a vegetable that is important to the economy of the world and Thailand. In 2020, Thailand had 37,420 rai of tomato harvested area with a total yield of 132,650 tons (OAE, 2020). Tomato is also important to health due to consumers are paying more attention to health concerns. The cherry tomato (*Lycopersicon esculentum* var. cerasiforme) is small-fruited; when fruits are ripe, the fruit color turns red, yellow, or orange according to the species. Ripen fruits are preferably eaten fresh, with firm flesh, sweet and sour tastes (Thepnarong, 2013). Tomato ripens fruits are a good source of carotenoids, naturally occurring pigments commonly found in plants (Ketsakul, 2015). In addition, lycopene is an important compound in the carotene family, found predominantly in red tomatoes (Stahl and Sies, 1996). The demand for healthy tomato markets has increased for consumers. However, the tomato production side still faces technical problems, especially under conventional field conditions. For example, climate changes or high temperatures during the rainy season can cause lower fruit set and fruiting. Moreover, high moisture in the soil and air humidity are suitable for promoting some root diseases and severe outbreaks resulting in low tomato production and insufficient yield to be sold in the market.

The hydroponic system is a plant cultivation technique using only water with nutrient solutions or non-soil materials. This technique is an efficient system that can produce safe and stable yields; it is able to produce vegetables even during the period when planting condition in regular soil is quite difficult (Sritontip et al., 2017). In general, plant growth depends on several factors, one of which is plant nutrients. Moreover, the nutrient solution is the most important factor for plant growth, development, yield, and quality. The electrical conductivity (EC) in the nutrient solution concentration is an index of salt concentration that defines the total amount of salts in a nutrient solution. EC level in the nutrient solution is a good indicator of the number of available ions to the corp in the root zone that the higher and lower EC may severely affect plant growth, development, and yield (Trejo-Téllez and Gómez-Merino, 2012; Nemali and van Iersel, 2004) Nantakit (1995) reported that EC values were relatively influential in the growth and yield of plants, and appropriate EC values are required for individual plant needs. Crop yields will be decreased if the EC value is too low or too high.

Micro/nanobubbles (MNBs) technology has been developed in Japan with wide applications for agriculture and fishery due to their tendency to decrease in size; subsequently, collapse under water, and generate free hydroxyl radicals resulting in a significant increase in ion concentration that stable in water for a long period of time (Takahachi et al, 2007; Marui, 2013). The application of MNBs can be employed for a variety of purposes such as wastewater quality improvement, disinfection, stimulating seed germination, promoting the physiological activities of other organisms, etc. (Oshita and Liu, 2013). Phaengkieo et al. (2019) reported that MNBs with a hydroponics system promoted plant growth, canopy width, and root length and reduced planting time by about a week in lettuce. Ebina et al. (2013) experimented to compare the growth of lettuce grown in a hydroponic solution with or without using MNBs. Their data indicated that MNBs stimulated plant height, leaf length, and fresh weight. However, data is limited in referring to the effect of EC levels and MNBs on the cherry tomato. Therefore, this research aimed to evaluate the effect of EC levels and MNBs on the growth, yield, and leaf nutrient contents of cherry tomato under a hydroponic system.

MATERIALS AND METHODS

This study was carried out in a greenhouse environment from July to October 2021 at the Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Lampang province, Thailand. The experiment was assigned using a 3x2 factorial arrangement in a completely randomized design. Factors were factor A, with three levels of electrical conductivity (EC) at 1, 2, and 3 mS/cm in the nutrient solution of the hydroponic system, and factor B, with two levels MNBs, including with or without the application of MNBs, respectively. There were 10 replications of each method with one plant each. Cherry tomato seeds were germinated in 104-cell nursery seedling trays. When seedlings were 28 days of age, they were transplanted into a hydroponics system. All seedlings were grown in deep-flow technique (DFT) hydroponic. The U-shaped PVC containers were sized 34 cm in width, 3 m in length, and 12 cm in height (5 cm nutrient solution level height) and 100 liters for nutrient solution containers (Figure 1). The nutrient solution formula modified from Huett (2003) and Sritontip et al. (2017), the 1 liter of stock solution fertilizers consisted of stock fertilizers A and B. The stock fertilizer A contained 128 g $Ca(No_3)_2 \cdot 4H_2O$ and 56 g Fe-EDTA and the stock B fertilizer contained 8.7 g $NH_4 \cdot H_2PO_4$, 13.60 g KH_2PO_4, 133 g KNO_3, 51.80 g $MgSO_4$, 0.30 g $MnSO_4$, 0.20 g $ZnSO_4$, 0.035 g $CuSO_4$, 0.55 g HBO_3, and 0.0175 g $(NH_4)_2MoO_4$ and nutrient solution pH was adjusted to be 6.5 by addition of sulfuric acid (H_2SO_4). Then, stock nutrient solutions A and B were diluted in 100 liters of water and mixed to obtain designated levels of electrical conductivity in a nutrient solution container every week. For the application of MNBs, the MNBs generator model KVM-25 (developed by the Faculty of Engineering, RMUTL, Thailand) was used and applied for 10 minutes at 10.00 A.M. every two days. This could support a water flow rate of 25 L/min, an airflow rate of 2 L/min, an operating pressure of 0.25-0.4 MPa, and a 0.75KW pump; having air nanobubbles, the total bubble distribution was 2.3141E+11/ml, median size 87 nm, mode size 209 nm and average size 199 nm that measured by Horiba-960A laser scattering particle size distribution analyzer® (Figure 2). The EC was controlled with an EC meter model EC 59 (Martini Instrument, Romania).

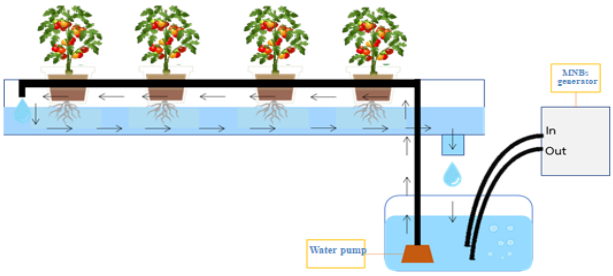

Figure 1. Hydroponic system model for growing the cherry tomato.

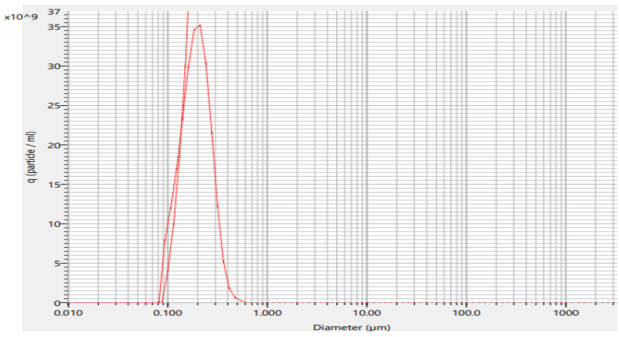

Figure 2. Diameter of micro/nanobubbles produced by KVM-25 detected by Horiba-960A.

The physiological character attributes of cherry tomatoes in a hydroponic system were measured, including stem height, inflorescence growth, leaf greenness, leaf chlorophyll fluorescence, yield, and fruit quality. Stem height (cm) was measured every week. The growth rates were

calculated according to the formula of Shabana et al. (1981) as follows:

$$\text{Growth rate} = \frac{(X_1 - X_0) \times 100}{X_0}$$

where: X_0 = the first measurement, X_1 = the next measurement.

For inflorescence growth, plants were measured in the length of inflorescence (cm), the diameter of inflorescence (mm), the number of leaves per inflorescence, leaf width (cm), and leaf length (cm). Changes in the greenness of the leaves were detected using the Konica Minolta model SPAD-502 plus® at 14, 21, and 49 days after transplant. Changes in the chlorophyll fluorescence of tomato leaves were measured by using Handy PEA$^+$® (Hansatech instruments, England) at 14, 21, and 49 days after transplant. Yield and fruit quality were recorded, including fruit width (mm), fruit length (mm), fruit weight (g), and yield weight per plant (g). Total soluble solids (TSS) were measured by extracting juice from cherry tomato fruit, dropped on a hand refractometer, and then reading the number of dissolved solids in degrees Brix (°Brix). The mature fully leaves at the 3rd leaf position of the floral shoot during the fruiting stage were collected to analyze for nutrient concentrations. The leaf samples were washed and dried at 70 ◦C for 48 hours and milled. The nitrogen (N) was determined using a micro-Kjeldahl digestion solution. The digested solution was diluted prior to colorimetric analysis using the indophenol reaction (Novozamsky et al., 1974). Phosphorus (P) was determined by dry digestion followed by the vanadomolybdate method (Walinga, 1995). The potassium (K), Calcium (Ca), and magnesium (Mg) were analyzed by dry digestion and atomic absorption spectroscopy (PinAAcle 900T, Atomic absorption spectrophotometer, PerkinElmer, Massachusetts, United States) (Kalra, 1998; Walinga, 1995).

Data were analyzed using the two-way Analysis of Variance. Mean comparisons were compared using Duncan's new multiple range tests, and significance was set at P-value less than 0.05.

RESULTS AND DISCUSSION

Stem height

The result showed that the EC factor (Figure 3a) did not affect tomato plant height between 14 to 21 and 42 to 49 days after transplanting (P>0.05). However, the EC of 3 mS/cm had shown the highest stem height of growth rate at 35 days after transplanting. Factor B (Figure 3b) found that there was no statistical difference between with or without MNBs between 14 to 49 days after transplanting (P>0.05). There was no interaction detected (P>0.05) between the two factors (Figure 3c). EC values tended to have a linear relation to the plant growth rates and imply plant needs in response to appropriate EC values. Crop yields will fluctuate if the EC value is inappropriate (Nantakit, 1995). The decrease of EC in nutrient solution reduces the plant height and diameter of the stem in tomatoes (Lu et al., 2022).

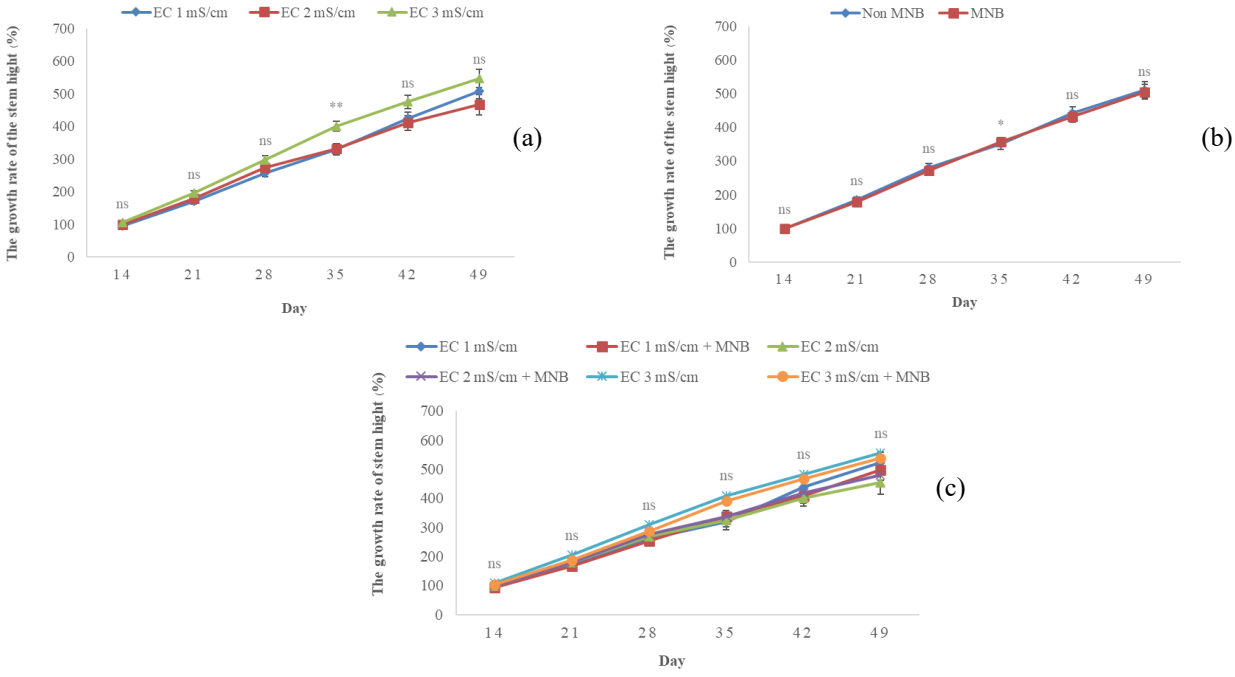

Figure 3. Stem height of cherry tomato from 14 to 49 days after transplanting under different EC levels (a), with or without MNBs (b), and interaction between EC and MNBs (c).

Inflorescence growth

Effects of EC and MNBs on the growth of the inflorescence, leaf number, and leaf size are shown in Table 1. In the flowering stage, the results indicated that EC and MNBs did not affect inflorescence length, inflorescence diameter, leaf number, and leaf width, except with MNBs showed higher leaf length than non-MNBs ($P<0.05$). However, there was no interaction between EC and MNBs factors or treatment combinations. This study suggested that EC levels and MNBs application did not affect inflorescence size and leaf number, and width of cherry tomatoes.

Table 1. Effect of EC and MNBs on the growth of the inflorescence, leaf number, and leaf size.

Items	Inflorescence length (cm)	Inflorescence diameter (mm)	Leaf number (leaves)	Leaf width (cm)	Leaf length (cm)
Factor A					
EC 1 mS/cm	153.85	8.13	16.95	3.55	7.65
EC 2 mS/cm	165.30	8.24	18.25	3.81	8.25
EC 3 mS/cm	164.65	8.24	15.60	3.64	7.84
F-test	ns	ns	ns	ns	ns
Factor B					
Non MNBs	156.67	8.12	17.33	3.61	7.59[b]
MNBs	165.87	8.29	16.53	3.72	8.23[a]
F-test	ns	ns	ns	ns	*
Interaction between factor A and factor B					
EC 1 mS/cm+ non- MNBs	149.90	8.11	17.80	3.61	7.22
EC 1 mS/cm + MNBs	157.80	8.15	16.10	3.49	8.07
EC 2 mS/cm+ non – MNBs	160.00	8.04	17.40	3.64	7.97
EC 2 mS/cm + MNBs	170.60	8.43	19.10	3.98	8.52
EC 3 mS/cm+ non – MNBs	160.10	8.20	16.80	3.58	7.57
EC 3 mS/cm + MNBs	169.20	8.28	14.40	3.70	8.11
F-test	ns	ns	ns	ns	ns
C.V. (%)	20.55	7.76	22.93	10.98	12.77

The values with the same letter within a column are statistically non-significant by Duncan's test at $P > 0.05$. The asterisk indicates significantly different means (*for ≤ 0.05, **for ≤ 0.01), otherwise not significant (ns).

Leaf greenness and chlorophyll fluorescence

Effects of EC and MNBs on leaf green color and chlorophyll fluorescence in different growth stages are presented in Table 2. During the vegetative stage, the interaction between EC and MNBs was observed in leaf green color and chlorophyll fluorescence ($P<0.05$). However, the interaction between EC and MNBs was observed only in the leaf green color ($P<0.05$), but not chlorophyll fluorescence ($P>0.05$). In the fruiting stage, no interaction was observed for leaf green color and chlorophyll fluorescence ($P>0.05$). In the vegetative stage, treatment combinations of EC at 2 to 3 mS/cm plus with or without MNBs showed the highest leaf green colors ($P<0.05$), while chlorophyll fluorescence was also affected by both EC and MNBs ($P<0.05$); ranged from 0.725 to 0.765 Fv/Fm ($P<0.05$). In the flowering stage, leaf green colors were the highest for treatment combinations of EC at 3 mS/cm plus with or without MNBs ($P<0.05$), but not chlorophyll fluorescence ($P>0.05$). In the fruiting stage, only EC at 2 to 3 mS/cm had significant effects on the leaf green color ($P<0.05$). No effects of MNBs were observed for chlorophyll fluorescence in the flowering stage**,** leaf green color, and chlorophyll fluorescence in the fruiting stage ($P>0.05$). Li and Stanghellini (2001) found that tomato trees received appropriate EC is beneficial for leaf chlorophyll content. However, the lower EC in the nutrient solution resulted in a decrease in the net photosynthetic rates and chlorophyll content of tomatoes (Lu et al., 2022).

Table 2. Effects of EC and MNBs on leaf green color (LGC) and chlorophyll fluorescence (CF).

Items	Vegetative stage		Flowering stage		Fruiting stage	
	LGC (SPAD)	CF (Fv/Fm)	LGC (SPAD)	CF (Fv/Fm)	LGC (SPAD)	CF (Fv/Fm)
Factor A						
EC 1 mS/cm	53.11^{b}	0.735^{b}	59.58^{b}	0.783^{b}	58.13^{b}	0.808
EC 2 mS/cm	59.95^{a}	0.747^{b}	61.22^{b}	0.806^{a}	61.17^{ab}	0.809
EC 3 mS/cm	60.91^{a}	0.770^{a}	67.19^{a}	0.800^{a}	63.22^{a}	0.811
F-test	**	**	**	*	**	ns
Factor B						
Non MNBs	57.68	0.746	62.36	0.796	61.02	0.809
MNBs	58.30	0.756	62.96	0.797	60.66	0.809
F-test	ns	ns	ns	ns	ns	ns
Interaction between factor A and factor B						
EC 1 mS/cm+ non - MNBs	53.26^{b}	0.725^{b}	59.71^{b}	0.784	58.39	0.808
EC 1 mS/cm + MNBs	52.96^{b}	0.745^{ab}	59.44^{b}	0.782	57.87	0.808
EC 2 mS/cm+ non - MNBs	58.83^{a}	0.746^{ab}	61.30^{b}	0.804	60.94	0.809
EC 2 mS/cm + MNBs	61.06^{a}	0.747^{ab}	61.14^{b}	0.808	61.39	0.808
EC 3 mS/cm+ non - MNBs	60.94^{a}	0.765^{a}	66.07^{a}	0.800	63.72	0.810
EC 3 mS/cm + MNBs	60.88^{a}	0.774^{a}	68.30^{a}	0.800	62.71	0.812
F-test	**	*	**	ns	ns	ns
C.V. (%)	7.25	4.21	7.25	3.10	8.78	1.05

LGC = leaf green color. CF = chlorophyll fluorescence. The values with the same letter within a column are statistically non-significant by Duncan's test at P > 0.05. The asterisk indicates significantly different means (*for ≤ 0.05, **for ≤ 0.01), otherwise not significant (ns).

Yield and fruit quality

The effects of EC levels and MNBs on yield and fruit quality are shown in Table 3. There were interactions between the two factors in all parameters of yield and fruit quality (P<0.05). The combination indicated that using EC at 1 to 2 mS/cm + MNBs tended to have higher means for fruit width, fruit length, and fruit weight (P<0.01) while EC 1 mS/cm without MNBs was the lowest (P<0.01). Using EC at 1 mS/cm + MNBs, and EC 2 to 3 mS/cm with or without MNBs tended to produce higher yields per plant of the cherry potato (P<0.05) while EC at 3 mS/cm with or without MNBs tended to obtain more amount of total soluble solids (P<0.01). Akrawong (2011) reported that the use of EC at 2.4 mS/cm in the pre-flowering stage and then increased to 3.6 mS/cm after the flowering stage increased fresh weight and plant dry weight of fresh edible tomato cultivar CLN 3125 after growing for 94 days. However, using EC at 1.2 mS/cm in the pre-flowering stage and increased to 2.4 mS/cm after the flowering stage resulted in higher fruit weight and total soluble solids. Tsutsumi et al. (2020) reported that aeration of the nutrient solution had been tried to enhance the growth of leafy vegetables such as lettuce, rape, and spinach in hydroponic cultivation. Ebina et al. (2013) found that the application of MNBs in lettuce grown in hydroponic systems increased vegetative growth when compared to no use of micro-nanobubbles. The application of MNBs can improve plant growth, yield, and fruit quality due to dissolved oxygen (DO) in nutrient solution increased when compared non-MNBs (Zhou, et al., 2019). Nevertheless, using EC at 3 mS/cm with and without MNBs did not affect yield and fruit quality except for total soluble solids because the higher nutrient concentration may cause irregular ion absorption of a root system. These results agree with Park et al. (2010) who reported that lower EC at 0.5 and 1.0 mS/cm produced higher leaf weight than those EC at 2 mS/cm, but in lettuce, while Zhou et al. (2019) reported that MNBs water oxygenation is an effective way to increase both yield and quality of tomatoes.

Table 3. Effect of EC levels and MNBs on yield and fruit quality.

Items	Fruit width (mm)	Fruit length (mm)	Fruit weight (g)	Yield per plant (g)	Total soluble solids (°Brix)
Factor A					
EC 1 mS/cm	22.03^{b}	34.32^{b}	9.23^{b}	2,357.85^{b}	5.21^{c}
EC 2 mS/cm	22.94^{a}	35.40^{a}	10.18^{a}	2,599.62^{a}	5.37^{b}
EC 3 mS/cm	22.01^{b}	33.97^{b}	8.98^{b}	2,526.25^{a}	5.62^{a}
F-test	**	**	**	**	**
Factor B					
Non-MNB	22.01^{b}	33.89^{b}	9.02^{b}	2,426.17^{b}	5.34^{b}
MNB	22.64^{a}	35.24^{a}	9.91^{a}	2,562.97^{a}	5.45^{a}
F-test	**	**	**	*	*
Interaction between factor A and factor B					
EC 1 mS/cm+ non - MNBs	21.52^{c}	33.14^{d}	8.52^{c}	2,262.54^{b}	5.14^{d}
EC 1 mS/cm + MNBs	22.54^{ab}	35.51^{ab}	9.94^{ab}	2,453.17^{ab}	5.27^{c}
EC 2 mS/cm+ non - MNBs	22.60^{ab}	34.56^{bc}	9.66^{ab}	2,499.49^{ab}	5.31^{c}
EC 2 mS/cm + MNBs	23.28^{a}	36.24^{a}	10.70^{a}	2,699.74^{a}	5.43^{bc}
EC 3 mS/cm+ non - MNBs	21.91^{bc}	33.97^{cd}	8.87^{bc}	2,516.49^{a}	5.58^{ab}
EC 3 mS/cm + MNBs	22.10^{bc}	33.97^{cd}	9.09^{bc}	2,536.00^{a}	5.65^{a}
F-test	**	**	**	*	**
C.V. (%)	3.87	4.06	12.10	10.36	4.91

The values with the same letter within a column are statistically non-significant by Duncan's test at P > 0.05.
The asterisk indicates significantly different means (*for ≤ 0.05, **for ≤ 0.01), otherwise not significant (ns).

Leaf nutrient concentration

There were interactions between EC and MNBs on concentrations of N, P, K, and Ca, except Mg in leaves (Table 4). The increase of EC at 3 mS/cm and with MNBs had significantly increased N, P, K, and Ca in leaves (P<0.05). However, EC 1 mS/cm+ non – MNBs was the lowest in N concentration (P<0.05). The experiment in the "Rinka409" tomato that was grown hydroponically showed that a high EC nutrient solution treatment led to an increase in the nutrient content in leaves (Suzuki et al., 2015). Maboko et al. (2017) reported that high EC in the cucumber experiment could enhance plant growth, leaf chlorophyll content, dry matter, and the increase in nutrient uptake of N, P, and K concentration in leaves. Data indicated that stimulation using MNBs in nutrient solution improved N, K, and Ca contents in tomato leaves because MNBs benefit from enriching dissolved oxygen (DO) in a nutrient solution, and nutrients are transported up by the root system (Park and Kurata, 2009; Park et al., 2010). However, Mg concentration in leaves was unaffected by EC plus MNBs (P>0.05).

Table 4. Effect of EC levels and MNBs on leaf nutrient concentration

Items	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Factor A					
EC 1 mS/cm	1.53^{c}	0.14^{b}	1.59^{b}	2.07^{b}	1.00^{b}
EC 2 mS/cm	1.85^{b}	0.14^{b}	1.66^{b}	2.29^{b}	1.07^{ab}
EC 3 mS/cm	2.24^{a}	0.16^{a}	1.83^{a}	2.74^{a}	1.11^{a}
F-test	**	*	**	**	*
Factor B					
Non-MNB	1.72^{b}	0.14	1.65^{b}	2.22^{b}	1.04
MNB	2.03^{a}	0.15	1.73^{a}	2.51^{a}	1.08
F-test	**	ns	*	**	ns
Interaction between factor A and factor B					
EC 1 mS/cm+ non – MNBs	1.38^{c}	0.13^{c}	1.58^{c}	1.90^{c}	0.97
EC 1 mS/cm + MNBs	1.69^{b}	0.14^{bc}	1.60^{bc}	2.23^{bc}	1.04
EC 2 mS/cm+ non – MNBs	1.76^{b}	0.13^{c}	1.63^{bc}	2.26^{bc}	1.06
EC 2 mS/cm + MNBs	1.93^{b}	0.14^{bc}	1.68^{bc}	2.32^{b}	1.08
EC 3 mS/cm+ non – MNBs	2.02^{b}	0.16^{ab}	1.73^{b}	2.51^{b}	1.10
EC 3 mS/cm + MNBs	2.46^{a}	0.16^{a}	1.92^{a}	2.97^{a}	1.12
F-test	**	**	**	**	ns
C.V. (%)	18.26	14.13	8.33	16.75	11.15

The values with the same letter within a column are statistically non-significant by Duncan's test at P > 0.05. The asterisk indicates significantly different means (*for ≤ 0.05, **for ≤ 0.01), otherwise not significant (ns).

CONCLUSIONS

The application of EC at 1 to 3 mS/cm did not affect the growth of the inflorescence, leaf number, and leaf size for cherry potatoes, but MNBs increased leaf length. However, the interaction between EC and MNBs occurred for leaf green color and chlorophyll fluorescence in the vegetative stage, leaf green color in the flowering stage, yield, and fruit quality. In the vegetative stage, treatment combinations of EC at 2 to 3 mS/cm plus with or without MNBs showed the highest leaf green colors, while chlorophyll fluorescence was also affected by both EC and MNBs. High EC plus MNBs increased N, P, K, and Ca in tomato leaves. Results from this experiment suggest that levels of electrical conductivity and micro/nanobubbles have the potential to improve some growth parameters, yields, and fruit quality of cherry tomatoes.

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Cooling Holstein cows and heifers before parturition during summer: physiological responses prepartum and productive responses postpartum

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ABSTRACT

The aim was to compare physiological parameters prepartum and productive responses postpartum of multiparous cows, and first-calf heifers cooled 30 d before parturition. Twelve cows and twelve heifers were subjected to a cooling system for 30 d before the predicted calving date. Before calving, respiration frequency (RF) was measured twice a day (AM and PM) once a week; also, a blood sample was obtained from the coccygeal vein once a week for a biochemical profile. After calving, productive parameters measured were calf birth weight and growth until weaning, colostrum, milk quality, and milk production. Mature cows had 6.5% less RF ($P<0.05$) than heifers during the morning, but this difference increased to 23% during the afternoon ($P<0.01$). Mature cows exhibited higher ($P<0.05$) mean corpuscular volume, mean corpuscular hemoglobin, and platelet distribution width than heifers; however, heifers showed higher ($P<0.05$) red blood cell count than mature cows. In comparison, colostrum fat was higher ($P<0.05$) in heifers, protein, SNF, and density were similar between groups. Milk quality did not differ between cows and heifers, but milk yield at 30, 60, 90, 120, and 150 d was higher ($P<0.05$) in mature cows than in heifers. Calf mortality, calf birth weight, body weight at 30 and 60 d, as well as daily weight gain at 60 d, were similar in both groups of mothers. In conclusion, mature cows showed better physiological and productive responses than heifers when cooled with spray and fans for 30 d prepartum under hot and dry stressful conditions.

Keywords: heat stress, dry period, milk production, colostrum quality, calf birth weight

INTRODUCTION

Climate change is associated with an increasing world temperature; according to climatic predictive models, by the year 2100, the mean global temperature will increase from 1.1 to 6.4°C (Hansen et al., 2010). Weather changes include more extended and more intense heat weaves during summer months, negatively impacting on agriculture and animal husbandry, especially those located in arid zones. As a result, summer heat stress (HS) produces hyperthermia in domestic animals reared outdoors and represents a critical challenge that the livestock industry has been facing with more emphasis in recent years (Sejian et al., 2018). In fact, the annual economic losses of dairy cattle originating from the effects of HS in the United States of America were estimated at about 900 million dollars, which included lower milk production, reduced reproductive rates, and depressed immune function (St-Pierre et al., 2003).

Most of the research on the evaluation of the effects of HS in dairy cattle has been completed during the lactation period of the cows, so they do not consider the dry period, that is, the last 60 d before parturition. The prepartum period is essential for the lactating cow. It has a significant impact not just on early lactation, but on the entire lactation period, as well as on the development of the newborn (Karimi et al., 2015). Several stressors occur in the dairy cow when she transits from the non-lactating to the lactating state, resulting in production and welfare impairments to the cow and its offspring. The presence of HS during the dry period of Holstein cows resulted in diminished milk production in the subsequent lactation at different stages and dysfunction of the immune system (Tao et al., 2012).

The mammary tissue during the dry period experiences extensive growth and cellular turnover that can be affected by HS conditions. Limited cooling during the dry period (shade or intermittent soaking) has shown to be ineffective since just modest increases in milk production have been reported (Collier et al., 1982; Avendaño-Reyes et al., 2008). However, when the cooling applied to dry cows is ampler (shade plus water and forced ventilation), milk production post-partum increased significantly. Tao et al. (2011) found that prepartum-cooled cows had more significant insulin resistance in peripheral tissues to direct more glucose to the mammary gland during early lactation compared to prepartum non-cooled cows, which suggests that this is a secondary metabolic pathway in which cooling cows during the dry period improves post-partum milk production. So the dry period is critical for maximizing milk yield and quality in the subsequent lactation.

In addition to these adverse effects of prepartum HS on dairy cows, colostrum quality and fetal growth are also affected. Cows that were non-cooled during the prepartum period exhibited lower serum IgG concentrations and efficiency in their absorption compared with cows that cooled during the same period (Tao et al., 2012). Consequently, when colostrum from cooled cows during the dry period was given to newborn calves, they showed higher blood IgG levels than those of non-cooled cows (Stott, 1980). Exposing Holstein heifers to HS before calving, Nardone et al. (1997) found that Ig concentrations in the colostrum were lower than their counterparts under thermoneutrality. Maternal HS reduces the birth weight of newborn calves, which reveals impaired fetal development in utero (Tao and Dahl, 2013). However, results on cooling first lactation cows before parturition during summer have yet to be studied. Therefore, the objective of this study was to compare some physiological and productive responses of first calf heifers and mature cows during the pre-and post-partum periods, which were cooled 30 d prepartum during summer in a hot and arid region.

MATERIALS AND METHODS

The care and management of the cows and heifers during the present study followed the procedures accepted by the Mexican Official Norms (NOM-051-ZOO-1995: humanitarian treatment of animals during mobilization).

Location of the study, experimental animals, and treatments

The study was carried on at a commercial dairy herd located in the rural community Ejido Jalisco, 27 km NW from the capital city of Mexicali, Baja California, México. Its geographic location is 115° 23′ longitude and 32° 52′ latitude, and it has 85 mm of average annual precipitation, 5 m above sea level, and an average yearly temperature of 24°C; this province is part of the ecosystem the Sonoran Desert and has a climate extremely arid, including maximum and minimum temperatures of 49 and -2°C during summer and winter, respectively (INEGI, 2017). The dairy herd milked around 600 cows. Twelve mature cows (4 to 6 years old) and twelve first-calf heifers (2 to 3 years old), all Holstein breeds, were used in the present study. All females were diagnosed as pregnant and had around eight months of gestation at the start of the study. Hence 30 d before their projected calving date, animals were assigned to one of two treatments: 1) Corral of mature cows with a cooling system under the shade, and 2) corral of first-calf heifers with the cooling system under the shade. All cows were fed twice a day at 07:00 and 14:00 h. A diet consisted of a mixture of two forages, oat straw, and sudangrass, given at 3% of the body weight (approximately 14 kg DM/cow). Fresh water was available all the time, having each corral having two waterers.

Corrals and cooling system

Two pens were used during the study, one for cows and one for heifers. The corral of the mature cows had an area of 2000 m^2 (50 x 40 m), while the corral of the heifers was 1443 m^2 (39 x 37 m). Each corral was equipped with a cooling system based on spray and fans. The fans had 76 cm of diameter (Universal Fog Cooling System®, Mesa, AZ, USA), 1 HP motor potency, and produced 15,000 CFM. They were installed at 2.50 m height and separated at 1.40 m each. A mature cow´s pen had five fans, and a heifer´s pen 3. A nylon high-pressure tubing line (Universal Fog Cooling System®, Mesa, AZ, USA) with sprayers at 1.35 m of separation was installed in front of the fans on each corral. The cooling system was activated with a thermostat set to operate when the ambient temperature reached 30°C.

Climatic variables

Climatic information was provided by the National Meteorological Service, a climatic station from the national weather network service in the state of Baja California, México. The climatic variables collected were ambient temperature (AT, °C) and relative humidity (RH, %) every 15 min, which were used to construct the temperature-humidity index (THI) following the formula proposed by Hahn (1999): THI = 0.81 (AT) + RH (AT - 14.4).

Physiological variables collection and analysis

Respiration frequency (RF) was recorded, registering the number of breaths during 30 seconds and multiplying this amount by 2 to obtain this variable in breaths per minute (bpm). This procedure was performed in all animals twice a day, twice a week, between 06:00 - 07:00 h (AM) and 15:00 - 16:00 h (PM) during the 30 d prepartum blood samples were collected weekly from the coccygeal vein in all females during the morning before the first feed was served. On each bleeding, two blood samples were collected, one in 10-ml vacutainer tubes containing EDTA, and the second in 5-ml tubes. The 10-ml samples were centrifuged at 3500 x *g* for 15 min at 10 ºC. Serum was stored at -20 ºC into 2-ml vials for determination of metabolites (i.e., glucose, cholesterol, triglycerides, urea, and total protein) in a blood auto-analyzer of liquid phase (EasyVet, KONTROLab, Morelia, Michoacán, Mexico). The second vial was used for the determination of electrolytes (i.e., sodium [Na^+], potassium [K^+], and chlorine [Cl^-]) using an electrolyte analyzer (LW E60A, LandWind, Shenzhen, China). Furthermore, the 5-ml tubes were used to analyze the hematological profile with the fresh blood samples in a blood auto-analyzer (Auto Hematology Analyzer, Mindray, BC-2800 Vet; Shenzhen, China). The hematological profile included the variables red/ blood cells (RBC), red blood cell distribution width (RDW), white blood cells (WBC), hemoglobin (HGB), monocytes (MON), lymphocytes (LYM), granulocytes (GRAN), procalcitonin (PCT), hematocrit (HCT), platelet distribution width (PDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), and mean platelet volume (MPV).

Production variables collection and analysis

Colostrum was collected after calving (0 h), 12, and 24 h postpartum. This sample was obtained from the four quarters of each animal and mixed in one sample for colostrum quality analysis which includes percentages of fat, protein, solids non-fat, and density. These determinations were performed in a LactiCheck LC-01 Milk Analyzer (Page & Pedersen International Ltd®, Hopkinton, MA, USA). Daily milk production was obtained by adding the volume of milk produced by each cow and heifer in the two milkings (04:00 and 16:00 h) per day. This information was collected from the electronic milking system of the dairy farm (DeLaval Herringbone Parlor HB50, Kansas City, Missouri, USA) using its supplementary software DeLaval DelPro™. Afterward, a milk sample from the four quarters of each animal was collected every week post-partum and mixed in one sample from morning and afternoon milking to be analyzed for the same milk quality parameters as colostrum until week four. Milk production was continuously recorded, and averages were calculated on days 30, 60, 90, 120, and 150 postpartum.

Statistical analysis

Respiratory frequency, metabolites, electrolytes, and hematological profile were analyzed with a 2 x 4 factorial arrangement in a completely randomized design with repeated measures, where factors were treatment (cows and heifers) and time (4 weeks). These models were performed with PROC MIXED of SAS (SAS, 2004), and the commands LSMEANS and PDIFF were used to estimate the least square means declaring significance at a 5% level. Colostrum and milk component variables were analyzed under a 2 x 3 or a 2 x 4 factorial arrangement in a completely randomized design, respectively. Factors of these models were treatment and time; the factor time had three levels for colostrum (0, 12, and 24 h post-partum) and four levels for milk production (7, 14, 21, and 28 d post-partum). Cow within treatment was the random effect, and the model was performed under a repeated measurement statement. When the factor time was significant, orthogonal polynomials were performed to determine the trend in the milk component as a function of time. Finally, milk production was analyzed with a 2 x 5 factorial arrangement in a completely randomized design, being the factors of treatment and time (30, 60, 90, 120, and 150 days post-partum). The PROC MIXED from SAS (SAS, 2004) was used, and the least square means were obtained with the PDIFF command. Significance was declared at a 5% level and a tendency between 5 and 10%.

RESULTS

Figure 1 shows the average of the climatic variables AT, RH, and THI during the 30 d before the calving date. The interaction treatment x time was significant ($P < 0.05$) for the variables of respiration frequency and milk production; however, the remaining variables were nonsignificant, so they are explained under these results. The averages of RF were 4.3 bpm higher ($P > 0.05$) during the morning, and this difference increased to 24.6 bpm ($P < 0.05$) during the afternoon in favor of heifers (Figure 2). The hematological profile of cows and heifers under cooling for 30 d before calving is presented in Table 1. The mature cows had higher ($P < 0.01$) MCV, MCH, and PDW than younger cows; meanwhile, RBC was higher ($P < 0.05$) in heifers than in cows; the remaining blood components were similar

(P > 0.05) between both groups. The average serum concentrations of the electrolytes Na^+, K^+, and Cl^- did not differ (P > 0.05) between cows and heifers (Table 1). Milk production was higher (P < 0.05) in cows than in heifers at 30, 60, 90, 120, and 150 days postpartum (Figure 3). For colostrum and milk components, there were differences (P < 0.05) by each factor individually. In colostrum, heifers produced more (P < 0.01) fat in milk than cows; the remaining colostrum components were similar (P > 0.05) in both female groups (Table 2). From calving to 24 h postpartum, colostrum protein showed a quadratic trend (P < 0.05) and density a linear trend (P = 0.0634) to reduce (Table 3). Milk fat and protein, as well as SNF, were similar (P > 0.05) in cows and heifers; however, SNF showed a trend (P = 0.0652) to be higher in heifers than in cows (Table 2). From day 7 to 28 postpartum (Table 4), milk fat showed a linear trend to decrease (P < 0.01), milk protein showed a quadratic trend (P = 0.0563), and milk SNF a trend to increase (P = 0.0996), while milk density a cubic response (P < 0.05). Calf birth weight in calves born from cows and heifers was similar (P > 0.05), as well as body weights at 30 and 60 d of age. Finally, calf mortality was similar (P > 0.05) in calves born from cows and heifers (Table 5).

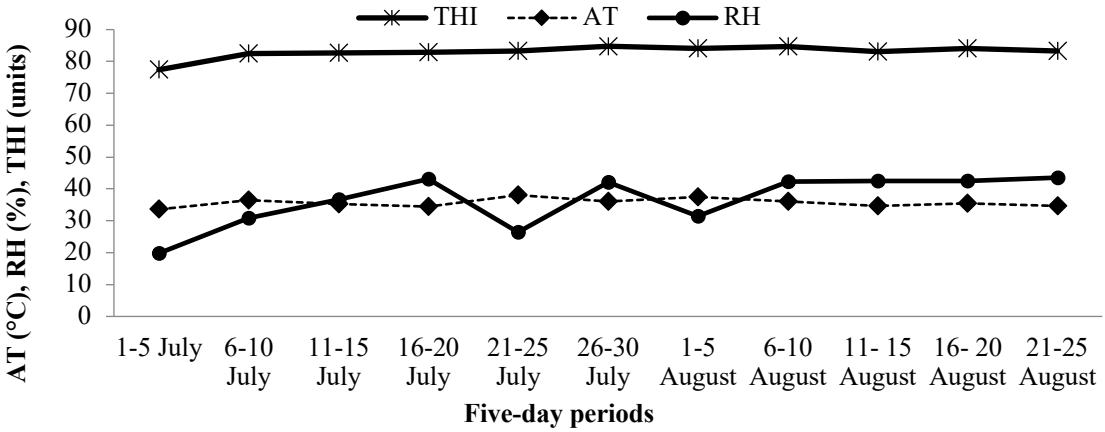

Figure 1. Average of climatic variables in five-day episodes during the prepartum period

THI= Temperature-Humidity Index [Units]; AT= Temperature of the dry bulb [°C]; RH= Relative Humidity [%].

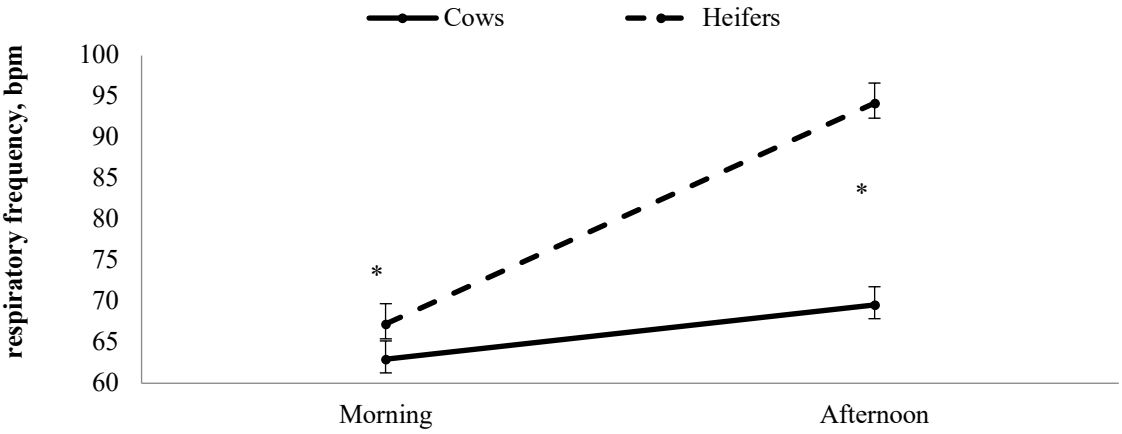

Figure 2. Averages of respiratory frequency (breaths per minute, bpm) during the morning and afternoon in cows and heifers under a cooling system 30 d prepartum [* (P<0.05)].

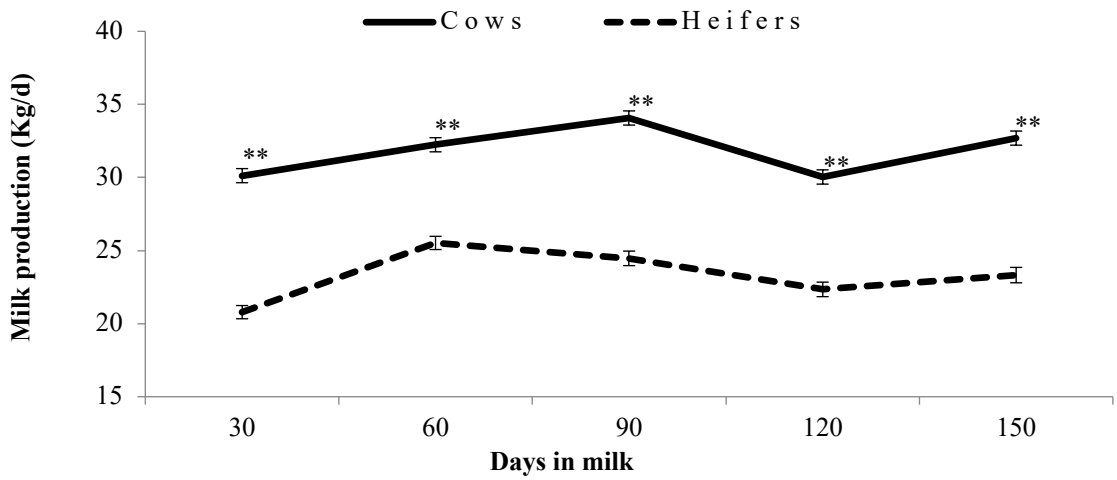

Figure 3. Milk production from day 30 to 150 in cows and heifers under a cooling system 30 d prepartum (** (P<0.05)].

Table 1. Hematological components and electrolyte concentrations in serum (mmol/L) of cows and heifers under a cooling system 30 d prepartum.

Parameter	Treatments		SEM	*P-value*
	Cows	Heifers		
Hematological				
MCV, fL	52.727	45.853	0.893	0.0001
MCH, pg	16.903	14.740	0.268	0.0001
MCHC, g/dL	32.192	32.168	0.200	0.9371
HCT, %	0.308	0.315	0.006	0.4710
PDW, %	16.710	16.280	0.112	0.0148
RBC, x12/L	5.862	6.884	0.169	0.0002
RDW, %	0.167	0.167	0.002	0.9848
WBC, x12/L	12.393	10.675	1.063	0.2885
HGB, g/L	9.910	10.084	0.190	0.5385
PLT, x12/L	369.29	352.77	20.438	0.5930
MPV, fL	5.157	4.998	0.065	0.1105
MON, x12/L	0.085	0.005	0.004	0.3231
LYM, x12/L	0.492	0.091	0.027	0.4063
GRAN, x12/L	0.425	0.452	0.026	0.4817
PCT, %	0.002	0.002	0.001	0.1649
Electrolytes				
Na^+	133.194	139.512	3.654	0.2519
K^+	4.825	5.147	0.317	0.4990
Cl^-	115.663	118.388	3.701	0.6239

MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; HCT = Hematocrit; PDW = Platelet distribution width; RBC = Red blood cells; RDW Red blood cell distribution width; WBC = White blood cells; HGB = Hemoglobin; PLT = Platelets; MPV = Mean platelet volume; MON = Monocytes; LYM = Lymphocytes; GRAN = Granulocytes; PCT = Procalcitonin; Na^+ = Sodium; K^+ = Potassium; Cl^- = Chlorine; SEM = Standard error of the mean.

Table 2. Colostrum and milk quality components in cows and heifers under a cooling system 30 d prepartum.

Item	Cows	Heifers	SEM	*P-value*
Colostrum, %				
Fat	3.10	5.89	0.31	<0.0001
Protein	6.83	6.67	0.26	0.6593
SNF	17.41	17.90	0.86	0.6835
Density	64.96	60.85	2.68	0.2823
Milk, %				
Fat	5.06	4.14	0.09	0.1398
Protein	3.44	3.58	0.07	0.1867
SNF	8.69	9.41	0.27	0.0652
Density	28.89	30.65	1.08	0.2593

SEM = Standard error of the mean; SNF = Solids non-fat.

Table 3. Trend in colostrum quality (%) by the time of sampling in cows and heifers under a cooling system 30 d prepartum.

Item	Time of sampling			SEM	*P-value*	
	At calving	12 hpp	24 hpp		Linear	Quadratic
Fat	4.69	4.68	4.12	0.38	0.2956	0.5577
Protein	7.49	6.19	6.58	0.32	0.0500	0.0344
SNF	18.96	17.29	16.72	1.05	0.1397	0.6693
Density	69.74	58.00	60.96	3.28	0.0634	0.0727

SNF = Solids non-fat; SEM = Standard error of the mean; hpp = hours postpartum.

Table 4. Trend in milk quality variables (%) by day post-partum in cows and heifers under a cooling system 30 d prepartum.

Item	Day post-partum				SEM	*P-value*		
	7	14	21	28		Linear	Quadr.	Cubic
Fat	4.98	4.59	3.87	2.89	0.57	0.0003	0.4291	0.1570
Protein	3.50	3.26	3.55	3.62	0.11	0.4435	0.0563	0.0693
SNF	8.23	8.38	9.27	9.65	0.47	0.0996	0.2358	0.2087
Density	29.0	25.84	31.05	33.07	1.41	0.0205	0.0616	0.0366

SNF= Solids non-fat; SEM= Standard error of the mean.

Table 5. Body weight at different times and mortality of calves born from cows and heifers under a cooling system 30 d prepartum.

Item	Cows	Heifers	SEM	*P-value*
CBW, kg	37.13	33.75	1.73	0.1737
W30d, kg	44.44	44.54	2.65	0.9782
W60d, kg	64.40	69.01	4.39	0.4720
TDWG, kg	0.442	0.581	0.08	0.2619
CMORT, %	16.67	33.36	--	0.3707

CBW = Calf birth weight; W30d = Weight at 30 d; W60d = Weight at 60 d; TDWG = Daily weight gain at 60 d of age; CMORT = Calf mortality; SEM = Standard error of the mean.

DISCUSSION

Climatic variables

As consistent environmental management practice, the most common stage to cool cows is during lactation, which means that during the period previous to parturition, cows are not cooled, even if this period is spent during the hot summer months. If cows are not cooled during the dry period, it is logical to think that first-calf heifers won´t be cooled either during the prepartum period. However, research on this topic has confirmed that cooling cows during the prepartum period has shown several advantages compared to dry cows not cooled (Adin et al., 2009; Karimi et al., 2015). Cooling pregnant heifers should also be beneficial from the physiological and productive perspective. This study was conducted to compare physiological parameters prepartum and productive parameters postpartum between MC and FCH when cooled 30 days prepartum. The average AT and THI registered during the experimental

period were 36°C and 82 units, respectively, demonstrating the severity of HS in this arid region. Several researchers have mentioned that HS starts its detrimental effects on dairy cattle at THI > 72 units (Fuquay, 1981; Armstrong, 1994). However, more recent literature (Carabano et al., 2014; Herbut et al., 2018) states that selection for milk yield in Holstein cattle has made lactating cows more sensitive to hot weather, so the threshold of THI should be reduced to 68 units. The highest THI found in the present study was in the last week of July (26-30), where the THI reached an average of almost 85 units (84.8 units). Modern dairy practices during intensive hot temperatures have incorporated the model of cooling their cows using spray and fans. However, equalizing milk production in summer and winter has been a great challenge, particularly for dairy herds established in arid environments; that's why milk production becomes seasonal in hot regions and is a concern for the dairy industry located in the deserts.

Physiological variables

During the morning, first-calf heifers had a higher RF than adult cows during the last 30 d prepartum, even though this difference increased five times more in the afternoon. These discrepancies by the time of the day were probably caused by the fact that mature cows adapted more to the hot weather. Castro-Montoya and Corea (2021) reported that using ¾ Holstein x ¼ Brahman lactating cows, primiparous cows showed higher RF and rectal temperature than multiparous cows under tropical conditions. The averages of RF registered for heifers were similar to those reported by Marcillac-Embertson et al. (2009), who cooled first calf heifers during the prepartum period with sprinklers and fans; however, they were slightly higher than the RF of multiparous cows (Fabris et al., 2019). Similarly, González et al. (2016) found that first calf heifers cooled during the postpartum period showed more RF from 11:00 to 17:00 h compared to those non-cooled in the same period (64 vs. 83 bpm); this difference was also observed in multiparous cows, but with lower values of RF (55 vs. 73 bpm). In general, averages of RF were higher than 60 bpm, which indicates that the cows in the present study were under HS (Hansen, 2019). This situation could be related to the fact that the cooling time was ineffective in stabilizing body temperature in the cows since less than 60 bpm indicates a non-heat-stressed cow (Berman, 2005; Nienaber et al., 1999).

The hematological profile of dairy cows may change by several factors such as breed, age, physiological stage, and even environmental conditions at the time the sample is obtained (Bhan et al., 2012). However, all hematological parameters measured were within the normal ranges for dairy cows (Woods and Quiroz-Rocha, 2010). Gomes et al. (2013) provided evaporative cooling to cows during their dry period in the summer months, finding that they had a more significant blood count of leucocytes and a smaller proportion of T-lymphocytes compared to non-cooled cows. Ramírez-Iglesia et al. (2001) evaluated the hematology profile of the dairy breed Carora 15 d prepartum, finding that MCV, MCH, RBC, PLT, WBC, HGB, and PLT were similar to those observed in the present study; the authors found more variation in the estimation of these parameters during the postpartum period. On the other hand, Saeed et al. (2021) measured several hematological parameters in pregnant cows', subjected to hot climatic conditions and reported averages somewhat higher than those found in the present study, which could be attributed to the fact that their cows were not under a cooling system during summer conditions (THI = 77-81 units). Changes in the electrolytes Na, Cl, and K were minimal during the prepartum period in this study. Maintenance of electrolyte homeostasis during the late gestation period is essential for the proper development of the fetus and newborn calf, preparation of the mammary gland for the subsequent lactation, and reproductive system regeneration after parturition (Skrzypczak et al., 2014). In general, homeostatic mechanisms in adult cows are efficient enough to maintain concentrations of sodium, potassium, and chlorides within normal frames, which are attributed to a relatively stable osmotic pressure that provides electroneutrality of extracellular fluids (Meglia, 2004).

Productive variables

Colostrum from heifers contained, on average, 2.8% more fat than from multiparous cows, with no difference in the remaining colostrum components measured (protein, SNF, and density). Under thermoneutral conditions, colostrum quality from multiparous cows has been slightly better than that from primiparous cows because it has a higher amount of immunoglobulin, protein, total solids, and density (Fahey et al., 2020), whit a lower fat percentage (Aydogdu and Guzelbektes, 2018). High environmental temperatures during late gestation of first-calf heifers markedly affect the colostrum composition (total protein, fat, IgG, IgA, short and medium-chain fatty acids, and lactose) compared to heifers under thermal comfort (Nardone et al., 1997). Results of the present study agree with those found by Soufleri et al. (2021), who reported that colostrum from first parity cows had higher fat content than cows of greatest parities; also, they found that colostrum from cows calved in the spring season had higher fat percentage than cows that calved during summer and autumn seasons. Likewise, Sánchez-Castro et al. (2014) evaluated the colostrum quality of multiparous and primiparous cows in the same arid region of the present study, finding that although the colostrum volume was similar between both groups of cows, protein, solid non-fat, and total

immunoglobulin content was higher in multiparous cows. However, colostrum fat was higher in primiparous cows than in adult cows, which agrees with the present study.

Even though milk components were similar between primiparous and multiparous cows, milk production was consistently higher in multiparous cows from calving to day 150 of lactation. Miller et al. (2006) compared different indicators related to metabolic activity, apoptosis, and endocrine control of the mammary gland in primiparous and multiparous Holstein cows. Primiparous cows presented lower milk production at 10 and 50 days in milk than multiparous cows; however, at 250 d, milk yield was similar between both groups of cows. These results agree with those found in the present study. In addition, primiparous cows exhibit lower secretory activity and DNA levels in their mammary gland in early lactation. Pollott (2011) evaluated milk yield at different lactation lengths in Holstein cattle, indicating that adult Holstein cows commonly produce more than 10,000 kg of milk during a complete lactation, while primiparous cows reach 8,000 kg, so milk yield undergoes a trend to increase with age. Several differences are responsible for this difference in milk yield. Heifers in their first lactation are still growing and acquiring body maturation, which requires additional energy expenditure, so they cannot produce enough metabolites and hormone signals to promote milk secretion (Akers, 2017). Some heifers did not reach complete mammary cell growth, and milk production is a direct function of the amount and activity of the mammary epithelial cells present in the mammary gland (Neave et al., 2017). Heat stress during the dry period causes a decrease in feed intake, which compromises the efficiency of the mammary gland reducing milk yield and quality in the subsequent lactation (Tao et al., 2011). Even though primiparous and multiparous cows were cooled during the last 30 d prepartum, it is possible that this cooling period was not enough to help cows to elude the negative effects of heat stress. Under heat stress, Holstein cows cannot save energy because of the fast adipose fat mobilization that occurs after calving when they are experiencing a negative energy balance. Considering a poor feed intake and the increase of energy supply for all body functions, the direct energy supply to the mammary gland is minimized, causing a drop in glucose absorption and fewer precursors to generate lactose, so milk production decreases (Baumgard and Rhoads, 2013). Cooling during the prepartum period has been demonstrated to increase prolactin secretion, recovers energy production, and decrease oxidative stress, improving animal welfare and production (Tao and Dahl, 2013).

Weights of calves at calving and growth until weaning were similar between primiparous and multiparous cows in the present study. Typically, under thermoneutral conditions, primiparous cows experience shorter gestation lengths, lower calf body weights, and higher percent of dystocia than multiparous cows, factors that also negatively impact milk yield and the lactation curve (Atashi and Asaadi, 2019). Heat stress during gestation affects placenta development, causing retardation in fetal development. First-calf heifers calving during hot conditions has been shown to produce calves with lower weights and slower antibody absorption from colostrum, which may compromise their health and growth (Karimi et al., 2015). It is important to state that all these negative effects of calves born from mothers subjected to severe heat stress during the prepartum period may remain during several generations, intensifying the problem of poor health and production in animals during their late lactations (Skibiel et al., 2018). So cooling strategies for cows prior to calving become essential for dairy herds located in regions with problems of high environmental temperatures. It is important to remark that the summer in the study site was especially hot since ambient temperatures were consistently higher than 30°C; hence no time for relief from heat stress was possible for the cows and heifers, even during the night-time. This scenario suggests that cooling should be given the completely dry period, not only 30 d prepartum.

CONCLUSIONS

Providing cooling during 30 d prepartum during moderate to severe heat stress conditions was more effective for mature cows than for first-calf heifers. This is based on their lower respiration frequency, lower number of erythrocytes, and average mean corpuscular hemoglobin. Also, milk yield was pointedly higher in mature cows than in heifers. Cooling Holstein cows and heifers should be part of the standard management practices of dairy herds in hot regions.

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• Proceeding, symposia etc.

Pratt, A., Gilkes, R.J., Ward, S.C., and Jasper, D.A. 2000. Variations in the properties of regolith materials affect the performance of tree growth in rehabilitated bauxite mine-pits in the Darling Range, SW-Australia. In: Brion, A., and Bell, R.W. (eds) Proceeding of Remade Land 2000, the International Conference on Remediation and Management of Degraded Lands. Fremantle, 30 Nov-2 Dec 2000. Promaco Conventions, Canning Bridge. p.87-88.

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