Vol. 74 No. 6, 2021

ISSN 0019-5146 (Print)
ISSN 2454-2172 (Online)

INDIAN JOURNAL OF DAIRY SCIENCE

INDIAN JOURNAL of DAIRY SCIENCE

November - December 2021, Vol. 74, No. 6

an IDA publication

Regd. No. 15665/68
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Contents

ISSN 0019-5146 (Print)
ISSN 2454-2172 (Online)

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Printed and published by Mr. Gyan Prakash Verma and edited by Dr. (Mrs.) Bimlesh Mann on behalf of the Indian Dairy Association and printed at National Printers, B-56, Naraina Industrial Area, Phase II, New Delhi and published at IDA House, Sector-IV, R.K. Puram, New Delhi-110022.

Indian J Dairy Sci 74(6): 469-478
https://doi.org/10.33785/IJDS.2021.v74i06.001

INVITED REVIEW

Kefir – a fermented milk product beneficial for gastrointestinal health

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Received: 9 September 2021 / Accepted: 6 December 2021 / Published online: 22 December 2021

Abstract: Kefir is becoming increasingly popular as a result of new research into its health benefits. It is a fermented product which originated in the Caucasus Mountains of Russia. Kefir is produced by the action of bacteria and yeasts existing in symbiotic association in kefir grains. The composition of kefir varies according to the type of milk and the microbiological composition of culture types (kefir grain or commercial starter culture). Kefir's distinctive flavour is attributed by lactic acid, ethanol, carbon dioxide and other flavouring agents, such as acetaldehyde and acetoin. The microorganisms in kefir produce vitamins, degrade protein and hydrolyse lactose making it a highly nutritious and digestible product. Kefir has a long history of health benefits in Eastern European countries. In this review, manufacturing technologies, physicochemical properties, microbiological composition, therapeutic activities (viz. antimicrobial, anti-carcinogenic, immunomodulatory, probiotic properties etc.) as well as other health benefits, like reducing cholesterol and improving lactose tolerance have been summarised.

Keywords: Gastrointestinal benefits, Fermented milk product, Kefir, Therapeutic properties

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Introduction

From prehistoric time, the manufacture of acidified milk products have been known in all countries. Acidification mostly depends on lactic acid bacteria (LAB) naturally present in milk that yield lactic acid required for coagulation. When these LAB are accompanied by yeasts or moulds they provide distinct features to the cultured product. Researches all over the world revealed the significance of fermentation as a way of conservation, preservation, improvement of nutritive value as well as enhancement of sensory characteristics. Evidences show that *Kombucha* (fermented black or green tea drinks) manufacture dates back to approximately 220 B.C. (Dufresne & Farnworth, 2000), while recent proteomic analysis have shown that Kefir-like milk to have been fermented some 3500 years ago in Asia (Yang et al. 2014). The universal functional beverage market is a developing part of the food trade as present-day health-conscious end users show growing appetite for foods that can enhance well-being along with lowering the risk of diseases.

Kefir is an energising cultured milk beverage with an exotic sour and slightly alcoholic flavour. It is prepared by inoculating milk with kefir grains. These grains are small unevenly shaped, yellowish-white, granules similar to cauliflower florets. A typical kefir is fizzy and have pourable consistency. It is a self-carbonated beverage with a distinctive flavour due to a mixture of lactic acid, ethanol, carbon dioxide and other flavour compounds namely acetaldehyde , acetoin etc. There is a symbiotic relationship between bacteria and yeast (Vedamuthu, 1977). According to Koroleva (1988) yeasts in Kefir enhance the activity of the lactic acid bacteria by supplying them growth stimulants as well as processing some of the lactic acid.

Kefir – A brief history

Kefir has been originated from Turkish words "keyif" meaning pleasure and "kopur" meaning milk or froth. The herdsman of Caucasus discovered Kefir while transferring milk in leather pouches as the milk would ferment to develop into an effervescent and appetizing drink. Thus, they started making Kefir by adding Kefir grains to milk in leather pouches and hanging them near doorways thereby stirring the fillings when someone knocked

the bag while walking through the doorways. It is said that in the early part of the 20th century, a Russian lady named Irina Sakharova persuaded a prince in the Caucasus to provide her few Kefir grains. She began producing Kefir in Moscow and ever since then it has been a Russian primary food.

Kefir is a traditional fermented dairy product of Middle East. It originated from Caucasus Mountains in former Soviet Union, Central Asia. It is thick self-carbonated beverage with a smooth, marginally foamy body and whitish hue having very small portion of alcohol. It is produced by fermentation of milk with kefir grains or mother culture prepared from the grains. Kefir can be prepared from any type of milk such as cow, goat, sheep, coconut, rice and soy but commonly cow milk is used (Irigoyen et al. 2005). Apart from these, milk can be pasteurized, unpasteurized, whole fat, low fat, skim and no fat.

The following description of Kefir has been given by Codex Alimentarius: Starter culture prepared from Kefir grains, *Lactobacillus kefir*, and species of the genera *Leuconostoc*, *Lactococcus* and *Acetobacter* growing in a strong specific relationship. Kefir grains constitute both lactose-fermenting yeasts (*Kluyveromyces marxianus*) and non-lactose-fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae* and *Saccharomyces exiguus*). The pivotal indicator for sensory characteristics of Kefir is carbon dioxide (1-2g/l) which gives refreshing and slightly effervescent taste highly cherished by consumer (Beshkova et al. 2002). Irigoyen et al. 2005 stated that Kefir contains 0.08-2% (w/w) alcohol and about 4.5 pH. About 40 aromatic compounds namely diacetyl and acetaldehyde are present in Kefir giving its characteristic flavour and aroma.

Kefir manufacture

There are mainly two methods for manufacture of Kefir: traditional (authentic) and industrial (commercial) processing (Guzel-Seydim et al. 2010).

In the traditional method, Kefir grains are added directly to the pasteurized and cooled milk followed by incubation with stirring for approximately 24 h at 25 - 30 °C. Incubation temperatures above 30°C encourages the growth of thermophilic LAB but is a disadvantage for yeast growth and mesophilic LAB (Rattray and O'Connel, 2011). After fermentation, the grains are separated from the milk by filtering with a sterile sieve and can be dried at room temperature and kept at cold storage in milk for the next inoculation. In industrial process Lyophilized starter cultures containing LAB and yeast are used for inoculation (Guzel-Seydim et al. 2010). This is due to difficulties in post fermentation necessities of the Kefir grain separation at the end of fermentation. In this method, activated starter culture is added to homogenized and pasteurized milk containing 2–5% milk fat. Following fermentation at 25-30 °C for a period of 20–24 h, the product can be stored at refrigerated temperature up to 20 days (Guzel-Seydim et al. 2010).

The maturation consists of maintaining kefir at 8-10 °C for up to 24 h (Beshkova et al. 2002; Rattray and O'Connel, 2011) to allow microorganisms primarily yeast grow contributing to the particular flavour of the product (Beshkova et al. 2002). There is rise in dimension and amount of kefir grains about 5-7% (Leite et al. 2012) of the biomass throughout fermentation. These grains can maintain their activity for years when preserved carefully (Lopitz-Otsoa et al. 2006; Rattray and O'Connel, 2011). Mitra and Ghosh (2017) described the detailed production process of Kefir (Fig. 1).

The industrial manufacture of Kefir using grains as the starter culture is very difficult due to the complexity of their microbiological composition, which varies widely depending on the origin of the grains and conditions of storage and handling. Therefore, currently, there are commercial lyophilized starter cultures that mimic the microbial composition of the grains.

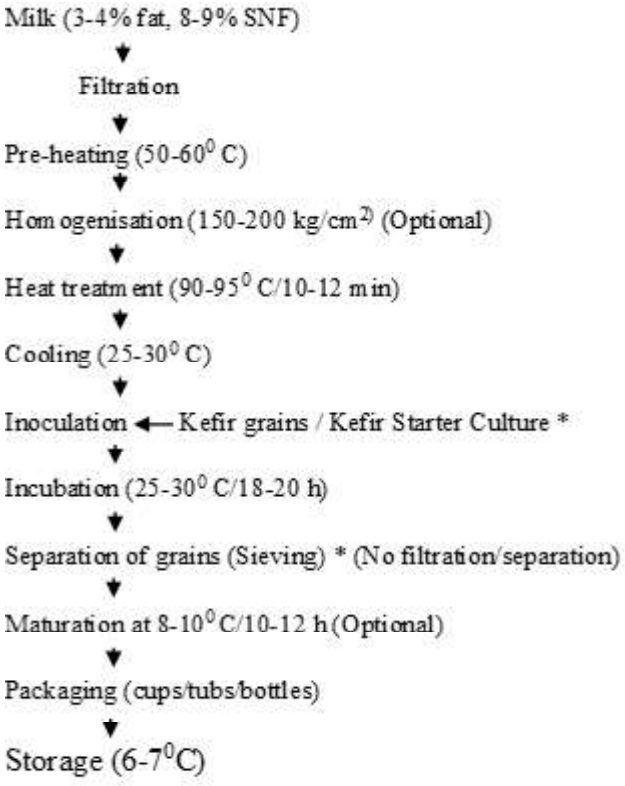

Fig. 1 Process flow for Kefir production

Chemical composition of Kefir

Investigators have perceived that composition of kefir products varies greatly and may be attributed to source and the fat content of milk, nature of the grains or cultures and the manufacturing

process. Fermentation produces amino acids such as valine, leucine, lysine and serine while alanine and aspartic acid increases as compared to starter milk (Table 1). Substantial quantities of pyridoxine, vitamin B_{12}, folic acid and biotin have been reported to be produced during kefir production, depending upon the origin of kefir grains whereas thiamine and riboflavin levels were reduced (Kneifel and Mayer, 1991).

The most important products formed during fermentation are lactic acid (0.8-1%), alcohol (0.01-2%), acetic acid (0.4 %) and CO_2 (1-4 mg/L). The ash content of Kefir samples was found to range from 0.55 to 0.66%. (Kök-Taş et al. 2014). More than 90% of lactic acid formed in Kefir is L (+) lactic acid. L (+) lactic acid is digested easily by the body and has physiological importance. Hydrolysis of lactose and enhanced microbial beta-galactosidase enzyme activity help lactose intolerant people who can consume Kefir easily. On the other hand, Kefir is a good source for B group vitamins, vitamin K and folic acid. Important aromatic compounds found in Kefir are diacetyl, acetoin and acetaldehyde. Diacetyl is harvested by *Streptococcus lactis* sp. *diacetylactis* and *Leuconostoc* sp. (Otles and Cagindi, 2003).

It was determined that Kefir increases secretion of some organs such as stomach and pancreas, and is helpful for nervous disorders, anorexia and insomnia. It was also found to be beneficial for high blood pressure, bronchitis and biliary disorders. Daily intake of half litre of Kefir for a day has positive effects for liver, gall and kidney functions in addition to stabilized effect on the metabolism. Kefir is effective for the functioning of kidneys, liver and nervous system because of it B vitamins content, and calcium and magnesium play role for the formation of healthy nervous system (Ahmed et al. 2013).

Sarkar (2007) indicated typical composition of Kefir as follows: 89–90% moisture, 0.2% lipid, 3.0% protein, 6.0% sugar, 0.7% ash and 1.0% each of lactic acid and alcohol while Beshkova et al. (2002) reported that Kefir contains 1.98 g/L of CO_2 and 0.48% alcohol. The amount of CO_2 in Kefir varies with the concentration of Kefir grains (10-100g/L) (Garrote et al. 1998).

In another study it was observed that Kefir grains contain 86.3% moisture, 4.5% protein, 1.2% ash and 0.03% fat (Liut kevicius and Sarkinas, 2004) while Brazilian kefir was found to contain 3.91% protein, 2.34% fat and 9.62% dry matter after 24 h of fermentation (Magalhaes et al. 2011).

Nutritional composition of Kefir

Table 1 Compositional details of milk Kefir

Components	100 g	Components	100 g
Energy	65 kcal	**Mineral content (g)**	
Fat (%)	3.5	Calcium	0.12
Protein (%)	3.3	Phosphorus	0.10
Lactose (%)	4.0	Magnesium	0.12
Water (%)	87.5	Potassium	0.15
Milk acid (g)	0.8	Sodium	0.05
Ethyl alcohol (g)	0.9	Chloride	0.10
Lactic acid (g)	1	**Trace elements**	
Cholesterol (mg)	13	Iron (mg)	0.05
Phosphates(mg)	40	Copper (mg)	12
Essential amino acids (g)		Molybdenum (µg)	5.5
Trytophan	0.05	Manganese (µg)	5.0
Phenylalanin+tyrosine	0.35	Zinc(mg)	0.36
Leucine	0.34	**Aromatic compounds**	
Isoleucine	0.21	Acetaldehyde	
Threonine	0.17	Diacetyl	
Methionine-cystine	0.12	Acetoin	
Lysine	0.27	-	-
Valine	0.22	-	-
Vitamins (mg)		-	-
A	0.06	B_{12}	0.5
Carotene	0.02	Niacin	0.09
B_1	0.04	C	1
B_2	0.17	D	0.08
B_6	0.05	E	0.11

Adopted from Otles and Cagindi (2003)

Vitamins B_5, B_2 and B_{12} are present in Kefir at approximate rate of 3, <5 and <10 mg/kg, respectively (Liut kevicius and Sarkinas, 2004). It also contains vitamins A, K and carotene (Table 1). Proteins present in Kefir are complete and partly digested aiding digestion by the body (Otles and Cagindi, 2003). During fermentation of Kefir, the amino acid profile changes leading to increased levels of threonine, serine, alanine, lysine and ammonia as compared to milk. Other amino acids namely valine, isoleucine, methionine, lysine, phenylalanine and tryptophan were also reported in Kefir (Otles and Cagindi, 2003; Sarkar, 2007).

In addition to basic nutrients increased level of amino acids, proteins, phosphorus, and calcium have been reported in Kefir making it an acceptable food product particularly in regions where it is consumed as staple food (Vinderola et al. 2004). Kefir contains phosphorus in abundance aiding in utilization of carbohydrates, fats and proteins for development, reconstruction and strength of cell (Otles and Cagindi, 2003). It is also a good source of calcium and magnesium. In one study Liut kevicius and Sarkinas (2004) examined the macro- and micro-elements in Kefir and showed that Kefir grains contain following macro-elements: potassium, 1.65%; calcium, 0.86%; phosphorus, 1.45%; and magnesium, 0.30% as well as micro-elements like (mg/kg) copper, 7.32; zinc, 92.7; iron, 20.3; manganese, 13.0; cobalt, 0.16; and molybdenum, 0.33.

Some important aspects on nutritional importance are summarized as follows.

Vitamin content

Some investigators are of the opinion that vitamins namely pyridoxine, vitamin B_{12}, folic acid, and biotin are produced in greater amount while a decline in thiamine and riboflavin content may occur during Kefir fermentation (Liut kevicius and Sarkinas, 2004). In addition to vitamin B-complex, substantial amount of vitamin K (Otles and Cagindi, 2003) and vitamin C (Khamnaeva et al. 2000) is also present in Kefir. Commercially accessible Kefir grains too tend to increase folic acid content of the product after fermentation (Alm, 1982).

Protein content

It has been well established that when Kefir grains were added as cultures in whey and soymilk, the protein content of the product increased as compared to whole milk. (FilChakova and Koroleva, 1997, Abraham and Antoni, 1999). Some scientists have reported proteolytic activity in Kefir (Yuksekdag et al. 2004a) which has been attributed to the presence of *lactococci* (Otles and Cagindi, 2003; Yuksekdag et al. 2004a).

Sugar content

Kefir contains about 6% sugar (Ozer and Ozer, 1999) and is known as kefiran which is a heteropolysaccharide, glucogalactan in

Fig. 2 Kefir grains

nature. It forms the key portion of gelatinous matrix containing microflora of Kefir. Rimada and Abraham (2006) has reported that kefiran enhances gel formation, rheology, and viscoelastic characteristics in gels produced by acidified milks and also produces gel at low temperatures. In addition to this it has also been stated that Kefir polysaccharides have numerous health improving properties namely inhibiting action on rotavirus (Song et al. 2007), immunomodulation, protection of epithelium.

Mineral content

A substantial content of major and minor minerals has been reported in Kefir namely calcium, potassium, phosphorus, magnesium and zinc, copper, manganese, iron, molybdenum, cobalt respectively. (Liut kevicius and Sarkinas, 2004).

Kefir grains

Milk is inoculated with Kefir grains for the preparation of Kefir (Fig. 2). They comprise of blend of microorganisms combined with casein and complex sugars by a matrix of polysaccharides called kefiran (Güzel-Seydim et al. 2005). Kefiran is a water-soluble branched glucogalactan (Leeet al. 2007) with hexasaccharide repeating unit composed of three glucose and three galactose residues (Maeda et al. 2004, Mukai et al. 1990).They are yellowish white elastic , slimy and irregularly shaped resembling cauliflower buds (Fig 2). Their size generally varies from 1-3 cm in length. (Farnworth, 2005 , Leite et al. 2013a). A unique symbiotic relationship exists between lactic acid bacteria (LAB), yeast (lactose-fermenting and lactose non-fermenting) and sometimes acetic acid bacteria in Kefir grains (AAB) (Farnworth 2005a; Leite et al. 2013a, b). Selected microorganisms from Kefir grains constitute Kefir starter.

Microbial composition of Kefir grains

Kefir grains have variable microbial composition which is governed by their geographic origin, climatic conditions, method

of manufacture of Kefir (time and temperature of incubation, agitation, grain to milk ratio) as well as the type of milk used for sub-culturing the grains (Filipcev et al. 2007; Dobson et al. 2011; Leite et al. 2013b). It has following chemical composition (% w/w): water (89–90), lipids (0.2), proteins (3.0), sugar (6.0), and ash (0.7). Preservation of these grains can be done by freezing, lyophilization, and refrigeration. Kefir grains microflora produce lactic acid, acetic acid, ethanol, peptides and other biologically active components thereby increasing the storage capability of milk and hindering the growth of detrimental and pathogenic microorganisms.

Lactic acid bacteria comprise 83–90% of the Kefir grains microflora while yeasts represent 10–17% with predominant lactose-negative yeast species (66–100%). Bergmann et al. (2010) counted the total average Lactobacillus as 8.5×10^5 CFU / g in Kefir grains. Kefiran a metabolite from Kefir, has been tested to have technological roles as a thickener, gelling agent and emulsifier (Farnworth, 2005a; Ahmed et al. 2013). Analysis of Kefir grains revealed the following bacteria: *Leuconostoc* ssp., *Lactobacillus lactis cremoris*, *Chyseomonas luteola*, *Acetobacter* and yeasts: *Sacharomyces cerevisae*, *Candida colliculosa*, *Toruspola delbruechii*, *Candida inconspicua*, *Candida magnoliae*, *Kloekera* sp., *Candida famata*, *Kluyveromices lactis*, *Kluyveromices marxianus* and *Candida kefir* are present in Kefir.

Moreover, the dispersal of microorganisms in Kefir grains is not equal. For example, while *Lb. kefir* strains are present on the outer surface of the grains, *Lb. kefiranofacies* are found in almost every region of the grains and the highest population is in the centre. Similarly, lactose-positive yeasts are detected mostly on the surface while lactose-negative yeasts at the centre. Contaminating microbes generally isolated from Kefir grains are *Geotricum, Pediococcus*, *Micrococcus*, *Enterococcus* and coliforms species (Seiler, 2003).

Kefiran is an exopolysaccharide component of Kefir that has significant importance in human health and nutrition and is recognized in most of the regions on this globe such as Central Asia, Southwest Asia, Japan, the Middle East, North America, Northern and Eastern parts of Europe, former USSR and North Africa (Koroleva, 1982; Otles and Cagindi, 2003; Piermaria et al. 2009). Especially in Soviet countries, Kefir has been used as prophylactic to lessen the threat of chronic diseases and has also been suggested for healing gastrointestinal diseases, IHD, allergy, and hypertension (St-Onge et al. 2000; Farnworth and Mainville, 2003). Bacterial population in Kefir ranges between 6.4×10^4 to 8.5×10^8 cfu/g, and for yeasts, it ranges between 1.5×10^5 to 3.7×10^8 cfu/g (Witthuhn et al. 2004).

The microbial population in Kefir grain was found to consist primarily of *lactobacilli* (65–80%) (Wouters et al. 2002), with *lactococci* and yeasts comprising the remainder. Witthuhn et al. (2004) reported that LAB and yeast level present in kefir grains vary widely, ranging from 6.4×10^4 to 8.5×10^8 and 1.5×10^5 to 3.7×10^8 cfu/mL, respectively. Irigoyen et al. (2005) reported that in addition to a viable population of 10^8 cfu/mL of *lactobacilli* and *lactococci* and 10^5 cfu/mL of yeasts, Kefir also contained 10^6 cfu/mL acetic acid bacteria after 24 h of fermentation. The amounts of yeast in Kefir vary from 10^3 to 10^6 (Grønnevik et al. 2011; Guzel-Seydim et al. 2005; Irigoyen et al. 2005; Simova et al. 2002). Grønnevik et al. (2011) found that the LAB count in kefir samples decreased during the first 4 weeks of storage, whereas yeast levels increased throughout the storage period.

Preparation of Kefir grains

The process for the preparation of Kefir has been described by Otles and Çagýndý (2003). A sterile water washed goat-hide bag filled with pasteurized milk and intestinal flora of a sheep was used. The temperature was maintained at 25°C for 2 days with every hour shaking. For a period of 12 weeks, three quarters of it was replaced with fresh milk immediately after coagulation. As a polysaccharide layer developed on the surface of the hide, it was removed and promulgated in pasteurized cow milk. This way kefir grains were developed and grown in fresh milk daily.

Activation of Kefir grains

Activation of lyophilized kefir grains can be done by inoculating sterilized skim milk (0.1% milk fat, 1L flask, 115 °C / 15 min) at 18–20 °C in 1:10 ratio followed by incubation for 24h with 3-5 mixings. After fermentation the grains were recovered by filtering the product and were used for next culturing after being washed with sterile water. (Simova et al. 2002). Cultivation of kefir grains in broth (distilled water with 5% brown sugar) was done through incubation at 25 °C for 24hr (Bergmann et al. 2010). Reactivation was followed by washing with distilled water and sub-cultivation in the same broth every 24 hours for a period of 15 days.

Preservation of Kefir grains

Freezing was considered as the best method for the preservation of Kefir grains (Garrote et al. 1997) besides lyophilisation can be done (dry/wet). Activity of dried grains last for about 12-18 months while for wet grains it is 8-10 days (Garrote et al. 2010). The disadvantage of this method is reduced lactose metabolism due to modification in bacteriological profile as compared to the original grains.

Kefir Culture versus Kefir grains

Several percentages of starter cultures isolated from the grains (LAB-Lactic Acid Bacteria, yeast, AAB-Acetic Acid Bacteria) were verified and it was found that traditional Kefir produced with Kefir grains was better acknowledged than kefir from starter culture (Assadi et al. 2000) while Carneiro (2010) developed a

starter culture from microorganisms isolated from Kefir grains and the product was more accepted than the traditional kefir.

Standardization of commercial production of kefir can be done using commercial cultures thereby allowing production of "kefir type" beverage with suitable flavour and good preservation properties (Beshkova et al. 2002; Carneiro, 2010). Commercial beverages may have commercial life up to 28 days while kefir prepared with grains must be consumed between 3-12 days (Leite et al. 2013b)

Therapeutic properties

The Kefir microbial metabolites and bioactive compounds are the outcome of yeast and bacterial action. The benefits associated with the consumption of Kefir reflected in the intestinal microbiota due to the inhibition of pathogens by acids and bacteriocin production in the intestinal mucosa.

Anticarcinogenic effect

In an experiment, an oral dose of 100 or 500 mg /kg of Kefir reported major reduction of tumour size in mice artificially transplanted with solid tumours of E-ascites carcinoma through stimulation of immunosuppressive action in spleen (Kubo et al. 1992). Strains of *Lactobacillus*, *Streptococcus*, *Leuconostoc* and *Streptococcus lactis* subsp. *cremoris* (Miyamoto et al. 1991) isolated from Kefir are capable of binding mutagens. However, Kefir from soymilk has been proved to be the best among all these (Liu et al. 2002).

Kefiran isolated from Kefir grains or harvested by *L. kefiranofaciens* has been reported to possess antitumor activity. It has been observed that sulphur containing amino acids play a key role in anticarcinogenic activity of Kefir and similar products (Guzel-Seydim et al. 2003). Liu et al. (2002) examined tumour inhibitory effect of Kefir prepared from soy milk and cows' milk with Kefir grains in mice. Both soy milk Kefir (70.9%) and cows' milk Kefir (64.8%) significantly inhibited tumour growth, compared with mice in the positive control group.

Antibacterial spectrum

The antibacterial properties of Kefir may be attributed to the integral formation of organic acids, hydrogen peroxide, acetaldehyde, carbon dioxide, and bacteriocins. It has been shown by Yuksekdag et al. (2004a) that 21 isolates of lactic acid bacteria from Turkish Kefir produced hydrogen peroxide in the range 0.04-0.19 µg/ml. Lacticin 3147, a bacteriocin produced by *Lactococcus lactis* strain DPC3147 from Kefir grains showed activity against *Escherichia Coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, *S. enteritidis*, *S. flexneri*, and *Yersinia enterocolitica* (Santos et al. 2003). Medrano et al. (2008) stated that 300-1000mg/L of kefiran from Kefir grains gave protection against damage to Caco-2 cells by *Bacillus cereus* B 10502

Table 2 Differences between Kefir and Yoghurt

Characteristics	Kefir	Yoghurt
Culture	Mesophilic	Thermophilic
Temperature	Moderate (25 – 30^{0}C)	Warm (42-46°C)
Milk	Works well with raw milk	Works well with pasteurized milk
Main metabolites	Lactic acid, CO_2, alcohol, Kefiran	Lactic acid
Colonization	Recolonization in gut with probiotics	No colonization in gut

Table 3 Differences between Kefir and *Dahi*

Characteristics	Kefir	*Dahi*
Culture	Kefir is prepared by fermenting milk with Kefir grains	*Dahi* is prepared by fermenting milk with *Dahi* culture
Texture	Uniform due to sieving	Grainy and sometimes pasty
Shelf-life	More	Less
Flavour	Yeasty with prickly sensation	Diacetyl flavour
Base medium	Milk/ Whey/Water	Milk
Maturation	Done to obtain typical flavour of kefir	Not done
Nature	Probiotic in nature	May or may not be probiotic unless probiotic organisms are added
Consistency	Mostly pourable	Pourable or set type
Immunomodulation	Kefiran is major immune modulating component produced by kefir grains	No specific immune modulating component is present

Kefir contains innate components from milk that hinder pathogens by primary and secondary metabolites namely small peptides, diacetyls, and organic acids harvested by microflora in Kefir (Golowczyc et al. 2008). Czamanski et al. 2004) reported that Kefir generally shows bacteriostatic effect on Gram-negative organisms however it has improved bactericidal effect against Gram-positive organisms. Kefir has shown antagonistic effect against *Listeria monocytogenes*, *Yersinia enterocolitica*, *Escherichia coli* (Gulmez and Guven, 2003), *Listeria innocua (*Morgan et al. 2000) and *Salmonella enteritidis* (Czamanski et al. 2004; Golowczyc et al. 2007). Undissociated acetic and lactic acid harvested by acetic acid bacteria and yeasts contribute to their bactericidal effect (Garrote et al. 1998). Besides LAB produces H_2O_2 which also lead to such diminishing effect (Yuksekdag et al. 2004a; Yuksekdag et al. 2004b)

Effect on immune system

Many researchers are of the view that Kefir may have immunomodulatory and prodigestive effect (Zhou et al. 2009). Vinderola et al. (2005) described that on consumption of Kefir both pulmonary and peritoneal macrophages lessen the pathogenic action there by affecting the mucosal response at various regions in the body in a murine (mice/ rodent) model. In another study an increased anti-cholera toxin (CT) IgA response was seen compared to controls in Kefir-fed young and old rats (6–26 months) through improved mucosal immune reaction.

Anti-inflammatory

Many studies have shown Kefir and its kefiran extract to possess anti-inflammatory activity. They exhibit this activity by preventing the formation of granuloma tissue (Rodrigues et al. 2005). They exhibit variable levels of anti-inflammatory activity such as in case of suspensions with molasses, fermented milk and kefiran extract an inhibition of 41, 44, and 34%, respectively have been reported.

Hypocholesterolemic effect

LAB present in Kefir can act on cholesterol through their metabolic products ensuring up to 30% binding of cholesterol. Vujicic et al. (1992) reported that 28-65% assimilation of cholesterol occurs when milk is inoculated with Kefir culture followed by incubation at 24.8°C for 24h. In another study Wang et al. (2009) have established cholesterol lowering activity of *Lactobacillus plantarum* MA2 isolated from Chinese Tibet Kefir. Liu et al.(2006a) stated that total cholesterol lowering properties of soymilk Kefir was found to be equally effective as milk Kefir and it also lowered serum triacylglycerol, and LDL cholesterol. All these studies reveal that Kefir and its components are prospective hypocholesterolaemic substance (Maeda et al. 2004; Liu et al. 2006a).

β-Galactosidase activity

β-galactosidase enzyme is naturally present in Kefir grains. It hydrolyses lactose making it suitable for lactose intolerant people (De Vrese et al. 1992). Hertzler and Clancy (2003) stated that a starter culture comprising of six bacteria (but not *L. acidophilus*) and one yeast produced Kefir which was equally effective in decreasing breath hydrogen in adult lactose maldigestions as compared to yoghurt. It has also been established that Kefir has some amount of β- galactosidase activity, converting lactose into easily digestible glucose and galactose thereby improving lactose tolerance.

Gastrointestinal proliferation

There is a wide believe that Kefir exerts prodigestive effect in gastrointestinal tract. Fil Chakova and Koroleva (1997) have stated that daily consumption of Kefir not only benefits gastrointestinal disorders but also helps in rapid healing in post-operative cases. Kefiran, exopolysaccharide of Kefir can alleviate the detrimental effect of *B. cereus.* In addition to this, it can control the virulence of microorganisms regarding intestinal infections (Medrano et al. 2008; Medrano et al. 2009).

Bacterial colonization

LAB from Kefir inhabitat in the intestine for brief period but produce favourable factors (probiotic). They are exceptionally tolerant to consecutive gastrointestinal tract situations. Santos et al. (2003) stated that 85% Lactobacillus species isolated from Kefir can stick to enterocyte like cells. Kefiran acts as a shield for LAB in hostile environments. Mitra and Ghosh (2019) incorporated probiotic organism of *Lactobacillus rhamnosus* GG along with kefir grains to manufacture and evaluated its quality characteristics for improved bacterial colonization .

Antiallergic properties

Daily intake of milk Kefir and soy-based Kefir products confine the IgE and IgG responses. Thus, gut microflora modification can be achieved with Kefir to inhibit food allergy and improve mucosal resistance in case of gastrointestinal pathogen infections (Liu et al. 2006b). In another study Lee et al. (2007) discovered that prevention of ovalbumin-induced eosinophilia in lung tissue and mucus hypersecretion can be achieved with Kefir thereby establishing itself as a potential therapeutic agent in management of allergic bronchial asthma.

Protection against apoptosis

Kefiran of Kefir have scavenging effect on superoxide radicals thereby giving protection against UV damage of human melanoma HMV-1 cells (Nagira et al. 1999). Matsuu et al. (2003) stated that milk Kefir reduced X-ray-induced apoptosis in rat's colon. In another study it was demonstrated that Kefir exhibited protection against apoptosis for colonic epithelial stem cells through termination of caspase-3 stimulation. Consumption of

Kefir may reduce the adverse side effects of irradiation in malignant patients undergoing irradiation therapy (Nagira et al. 1999; Matsuu et al. 2003).

Wound healing properties

It has been reported that the anti-inflammatory properties of polysaccharide present in Kefir extract may lead to the process of wound healing (Chena et al. 2008). Huseini et al. (2012) observed that lactic acid, acetic acid, polysaccharides and other chemicals present in Kefir were influential in wound healing. They also demonstrated that Kefir had improved wound-healing properties than conventional silver sulfadiazine treatment with respect to thermal injuries. Sugar prebiotics and peptide for example lactacin, bacteriocins, and kefiran are also present in Kefir (Schneedorf and Anfiteatro, 2004).

Antioxidant properties

Kefir samples were examined for antioxidant properties such as inhibition of ascorbate autoxidation, reducing activity, superoxide anion radicals and hydrogen peroxide scavenging activity. The maximum degree of inhibition was observed in whole soymilk Kefir. No hydrogen peroxide scavenging activity was displayed by the Kefir samples. The differences between Kefir & Yoghurt (Table 2) and Kefir & *Dahi* (Table 3) have been lighted.

Conclusions

In general, an increasing trend to consume nutritive foods possessing certain health benefits has been observed across the globe. This trend paved the way for regular consumption of cultured products like Kefir. Kefir has acquired increased preferences owing to neutraceutical and therapeutic potential. Several constituents like protein, vitamins, antioxidants, minerals, and certain metabolites contribute towards beneficial properties of Kefir. Numerous health benefits namely anticarcinogenic effect, gastrointestinal proliferation, antidiabetic properties, β-galactosidase activity etc. have been associated with Kefir. It has been reported (Koroleva, 1998) that kefir have been regularly used for managing numerous metabolic disorders, allergic disease, chronic enteritis etc. at hospitals of former Soviet Union. Consumption of Kefir has been advised for infants, children, women and individuals with lactose intolerance (Otles and Cagindi, 2003). Researches are in progress to identify organisms in complex microbiology of Kefir. Numerous bacteria from Kefir grains exhibited proteinase activity and certain bioactive peptides have also been identified in Kefir. Efforts have been made to industrially manufacture Kefir using starter culture isolated from Kefir grains. However, more research is required in this regard for obtaining traditional flavour along with other characteristics of Kefir.

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Indian J Dairy Sci 74(6): 479-485
https://doi.org/10.33785/IJDS.2021.v74i06.002

RESEARCH ARTICLE

Evaluation of selected physico-chemical, colour and textural characteristics of market *Gulabjamun*

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Received: 12 May 2021 / Accepted: 10 October 2021 / Published online: 22 December 2021

Abstract: *Gulabjamun* is one of the most popular traditional Indian dairy products. The various characteristics such as physico-chemical, colour and textural quality characteristics of six brands of *Gulabjamun* were investigated which plays important role in its marketing and consumer acceptance. The fat and unit-weight of market *Gulabjamun* were found statistically highly significantly different ($p<0.01$). The moisture content, percent absorbed sugar, GMD, sphericity of market *Gulabjamun* were found statistically highly significant ($p<0.001$). Apparent density was statistically significant ($p\leq0.05$). The fat, moisture and percent absorbed sugar of market *Gulabjamun* were ranged between 6.47±0.37 to 12.32±1.19 %, 40.51±0.70 to 47.53±0.42 % d.b. and 12.24±0.25 to 37.65±0.52%, respectively. The geometrical mean diameter, apparent density and unit-weight varied between 3.09±0.01 to 3.60±0.06 cm, 1.40±0.05 to 1.84±0.32 g/cm^3 and 24.21±0.29 to 44.40±0.45 g, respectively. Colour characteristics i.e. *L*, a*, b** and browning index (BI) market *Gulabjamun* were found statistically highly significant ($p<0.001$). The variation in the values of *L**, *a**, *b** and brownness index was observed to be between 34.70±0.41 to 46.46±0.55, 3.92±0.28 to 6.50±0.39, -2.30±0.59 to 11.48±0.48, and 2.77±1.33 to 36.98±0.56, respectively. The hardness, cohesiveness, gumminess, chewiness and resilience were found statistically highly significant ($p<0.001$) whereas springiness was found statistically non-significant ($p\geq0.05$). The hardness, springiness, gumminess, chewiness, cohesiveness and resilience ranged between 3.36±0.56 N to 10.71±1.66 N, 0.52±0.29 to 0.88±0.12, 0.86±0.13 N to 2.14±0.28 N, 0.44±0.24 N to 1.88±0.30 N, 0.18±0.01 to 0.25±0.01 and 0.24±0.01 to 0.35±0.02, respectively. It was observed that the size of market *Gulabjamun* (3.09±0.01 to 3.60±0.06 cm) may be taken as 3.0-3.6 cm for development of process equipments for *Gulabjamun* manufacturing.

Keywords: Colour, *Gulabjamun*, Market, Sphericity, Texture

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Introduction

India is the largest milk producing country in the world with about 19% contribution in total world milk production. The India's total milk production is estimated to have increased to 187.7 million tonne (MT) during year 2018-19 from 176.35 million tonnes during the year 2017-18 (NDDB, 2019). About half of total milk produced, is consumed in the liquid form and the remaining half is used for manufacturing of various milk products such as ghee, curd, butter, *khoa* and *khoa* based products, paneer, cheese, *chhana* and *chhana* based products, ice cream and milk powders. A traditional Indian dairy product (TIDP) refers to the products which are indigenous to India and some Asian countries such as Nepal, Bangladesh and Pakistan (Aneja et al. 2002). The various efforts towards its manufacturing including heat desiccation, heat coagulation, fermentation and use of different ingredients resulted into quality products and there are number of sweetmeats (TIDPs), their varieties and brands, available in the Indian market.

Gulabjamun is the fat deep fried and sugar soaked traditional dairy product popular throughout India. Traditionally, it is prepared by producing the homogeneous dough mass, by proper mixing and kneading of *Khoa*, *Maida*, and baking powder. Maida is used as binding agent which and it helps to prevent disintegration of *Gulabjamun* balls at time of frying. The balls prepared from dough are deep-fat fried in frying medium (ghee or refined vegetable oil) until a golden brown colour obtained and subsequently soaked into sugar syrup. Deep-fat frying is a very complex process. It is industrially important food processing operation which increases the acceptability of a food product. The fried food undergoes different properties changes like textural and chemical changes etc. The moisture and fat content of finished product significantly depends upon frying conditions

such as frying time and frying temperature. In general, the desirable characteristics of *Gulabjamun* are brown colour, smooth and spherical shape, soft and slightly spongy body (free from lumps), uniform granular texture with pleasant cooked flavour (Vasava et al. 2018). There are mainly two key processes, which are very important in *Gulabjamun* production i.e. frying of *Gulabjamun* balls and it's soaking in sugar syrup (after frying of balls). The deep fat frying and sugar soaking *of Gulabjamun* are most important with respect to its chemical, physical and textural properties.

The characteristics of market sample of various Indian dairy products have been reported by several researchers in published literature such as *paneer* (Rajorhia et al. 1984; Goyal et al. 2007; Desale et al. 2009; Godbole et al. 2013; Peter et al. 2015; Ranjan Naik et al. 2016) , *Rasogolla* (Desai et al. 1993; Srinivasa et al. 2017), brown peda (Londhe and Pal, 2008), *Halvasan* (Patel et al. 2010), *Dodaburfi* (Chawla et al. 2011), *KhoaJalebi* (Jayaraj and Pagote, 2012), *Thabdi* (Patel et al. 2012), *kheer mohan* (Meena et al. 2014) and *Khoa-peda* (Singh et al. 2018). However, the characteristics of market sample of *Gulabjamun* are rarely or not reported in published literature.

The characteristics of any food are very important parameters for food processing operation, heat and mass transport processes, food transport and handling, and for design of process, modelling and optimization. It will be useful for designing particular equipment or determining the behaviour of the product during its handling. The present study was undertaken to investigate the selected physico-chemical, colour and textural characteristics of the different brands of market *Gulabjamun.*

Material and Methods

Collection of Market Gulabjamun Samples

The various brands of *Gulabjamun* are being manufactured in different parts of India. These are being marketed throughout India in small tin cans or other appropriate packaging materials. For present study, different brands of *Gulabjamun*, manufactured in Nagpur (Maharashtra), Bengaluru (Karnataka), Bikaner (Rajasthan), New Delhi regions along with Karnal (local market), were procured from Delhi and Karnal market. The market Gulabjamun samples of different brands were collected as fresh as available in the market. The market samples of *Gulabjamun* were designated as M1, M2, M3 and M4 for established brands in India whereas M5 and M6 for sample from local market of Karnal, Haryana, India.

Physico-Chemical characteristics

Some physico-chemical characteristics, such as fat, moisture, percent absorbed sugar, geometrical mean diameter (GMD), sphericity, apparent density and unit-weight, of *Gulabjamun* were determined.

Fat

Fat content of the samples was estimated using Mojonnier method (BIS, 1981). The fat in the samples was calculated by following equation:

$$Fat\ (\%) = \left(\frac{W_d - W}{W_s} \right) \times 100$$

Where, W_s is the weight of the material taken initially for the test (g), W_d is the weight of the dish with the material after drying (g), and W is the weight of the empty dish (g).

Moisture

The moisture content in samples was estimated by the gravimetric method (AOAC, 2005). Following expression was used for determination of moisture on dry basis (% d.b.): ‘ ‘

$$Moisture\ (\%\ d.b.) = \left(\frac{W_s - W_d}{W_d - W} \right) \times 100$$

Where, W_s is the initial weight of the dish with sample (g), W_d is the final weight of the dish with the sample after drying (g), and W is the weight of the empty dish (g).

Percent absorbed sugar

Initial weight of *Gulabjamun* was measured using digital weighing balance. *Gulabjamun* was then placed on a plate and 5 kg weight was applied on plate. After 5 min, the weight of pressed or final *Gulabjamun* was taken. Percentage of absorbed sugar syrup was calculated using following equation (Mohanta, 2014).

$$Absorbed\ sugar\ (\%) = \frac{W_1 - W_2}{W_1}$$

Where, W_1 is the weight of *Gulabjamun* (g) and W_2 is the weight of *Gulabjamun* after weight is applied (g).

Geometrical mean diameter (GMD):

The major, intermediate and minor dimensions of *Gulabjamun* samples were measured as A, B and C, respectively by using a digital vernier calliper (make: Mitutoyo; range: 0-150mm; least count: 0.1 cm). The size of the *Gulabjamun*, in terms of geometrical mean diameter (GMD), was computed using standard equation (Srinivasa et al. 2017; Barnwal et al. 2017):

$$GMD = (A \times B \times C)^{1/3}$$

Sphericity

Sphericity (Φ) may be referred as the ratio of GMD to the major linear dimension, i.e., length (A). It was computed using the following equation (Srinivasa et al. 2017; Barnwal et al. 2017):

$$Sphericity, \phi = \frac{GMD}{A} = \frac{(A \times B \times C)^{1/3}}{A}$$

Apparent density (ρ_{app})

The apparent density (ρ_{app}) of the market *Gulabjamun* sample was computed as mass per unit volume from the following equation:

$$\rho_{app} = \frac{W}{\left(\frac{4}{3}\pi R^3\right)}$$

Where W is the weight of *Gulabjamun* (g) and R is the radius (0.5×GMD) of *Gulabjamun* (cm).

Unit Weight of the Gulabjamun

A precision electronic balance (model: KERRO BL 3003; capacity: up to 300 g; least count: 0.001 g) was used to measure unit weight of the market *Gulabjamun* (Srinivasa et al. 2017).

Colour characteristics

The consumer acceptability of *Gulabjamun* is very important and it depends upon the crust colour of it. The colour of market samples *Gulabjamun* was measured using a computer based image analysis technique (Minz et al. 2018). The sample was transferred into wide petri-dish and images of sample were captured in colour desk instrument which was based on reflectance spectroscopy technique. The digital images were processed using Scilab program to compute colour values. This colour value was obtained in terms of lightness, "CIE *L*" ranging from zero (black) to 100 (white), "CIE *a*" ranging from +60 (red) to -60 (green) and "CIE *b*" ranging from +60 (yellow) to -60 (blue). The browning index (BI) value of *Gulabjamun* was calculated using by following relation (Srinivasa et al. 2017; Barnwal et al. 2017).

$$BI = \left[\frac{100 \times (x - 0.31)}{0.17}\right]$$

$$\text{Where,} \quad x = \frac{(a^* + 1.75 \times L^*)}{(5.645 \times L^* + a^* - 3.012 \times b^*)}$$

Textural characteristics

Texture profile of *Gulabjamun* was determined by using texture analyser (TA-XT2i; M/s Stable micro systems; Software: Texture Expert Exceed, Version: 2.55), fitted with a 25 kg load cell and calibrated with 5 kg standard dead weight prior to use (Kumar et al. 2006). *Gulabjamun* was compressed twice in a reciprocating motion to obtain a two-bite texture profile curve using a double compression test. The various test parameters, used throughout the study, for whole, uncut *Gulabjamun* sample were P-75 compression probe, 1 mm/s probe pre-test speed, 0.5 mm/s test speed, 10 mm/s post-test speed, 7.5 mm distance (compression) and 25 ± 1 °C maintained sample temperature. The obtained texture profile curve (TPA) was used to determine the hardness, springiness, cohesiveness, gumminess, chewiness and resilience of the tested market *Gulabjamun* samples.

Statistical analysis

The experimental data for different characteristics such as physico-chemical, colour and textural characteristics were analysed statistically. The Analysis of Variance (ANOVA) for all various characteristics of market *Gulabjamun* were analyzed by SPSS (v. 15. 0) software and 2D-graphs of various characteristics were prepared by using Microsoft Excel-2007 software.

Results and Discussion

Physico-chemical characteristics of market *Gulabjamun* samples

The fat content of market *Gulabjamun* samples (M1, M2, M3, M4, M5 and M6) were found statistically highly significantly different ($p<0.01$) with each other (Table 1). It varied from 6.47±0.37% (M3) to 12.32±1.19% (M6). The fat content of *Gulabjamun* on drained weight basis was reported as about 10% (Aneja et al. 2002). The variation in fat content of *Gulabjamun*, prepared using khoa of Buffalo milk blending with sweet corn milk, was found to be 10.26-14.98% (Patil et al. 2017). The market *Gulabjamun* samples M5 and M6 were observed to be in nearly this range whereas M1, M2, M3 and M4 had lesser fat content. Minhas et al. (1985) reported that the fat content range 9.9 to 16.7% of *Gulabjamun* (prepared by different formulations mix, different frying temperature range and different soaking time range) was more than the fat content of studied market *Gulabjamun*. The moisture content of studied market *Gulabjamun* samples were found statistically highly significant ($p<0.001$) among each other (Table 2). The moisture content of samples M3, M4, and M5 were statistically at par with each other. The moisture content of M3, M5 and M6 were statistically at par with each other whereas that of M1, M3, M4 and M6 was statistically different with each other (Figure 1). It varied from 40.51±0.70 %d.b. (M6) to 47.53±0.42 %d.b. (M2). The variation of moisture content of *Gulabjamun,* prepared by using different

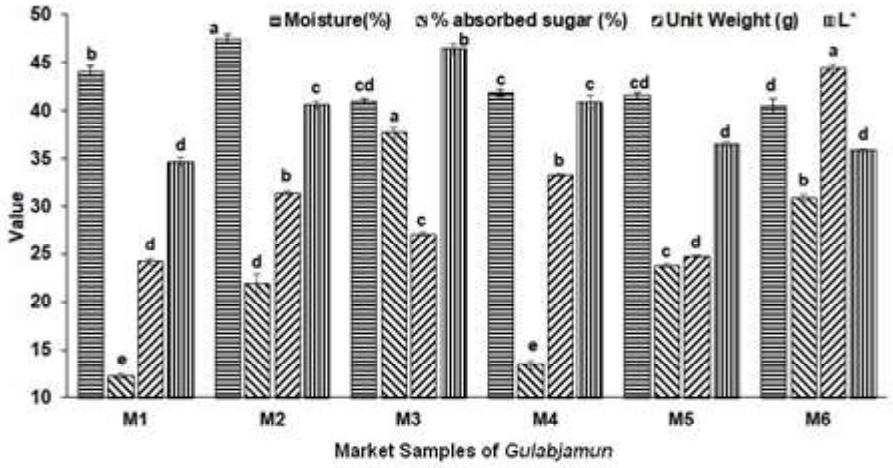

Fig. 1 Moisture, percent absorbed sugar, unit-weight and *L** values of Market *Gulabjamun*

Table 1 Fat, apparent density, b*, browning Index and hardness of Market *Gulabjamun*

Market *Gulabjamun*	Fat (%)	Apparent density (g/cm^3)	*b**	Browning Index	Hardness (N)
M1	8.53^{cd}±0.43	1.56^{a}±0.04	3.17^{c}±0.09	17.52^{c}±0.80	5.78^{bc}±0.28
M2	9.59^{bc}±0.27	1.84^{a}±0.32	5.63^{b}±0.27	26.21^{b}±1.32	7.15^{ab}±0.38
M3	6.47^{e}±0.37	1.57^{a}±0.03	11.48^{a}±0.48	36.98^{a}±0.56	10.71^{a}±1.66
M4	7.63^{de}±0.35	1.84^{a}±0.24	5.39^{b}±0.02	24.53^{b}±1.63	9.95^{a}±1.63
M5	10.29^{b}±0.37	1.40^{a}±0.05	-2.30^{d}±0.59	2.77^{d}±1.33	7.61^{ab}±2.22
M6	12.32^{a}±1.19	1.83^{a}±0.07	2.57^{c}±0.34	20.09^{c}±0.80	3.36^{c}±0.56
ANOVA for fat, apparent density, *b**, browning Index and hardness of Market *Gulabjamun*					
DF	5	5	5	5	5
SS	64.285	0.519	307.255	1918.404	109.474
MS	12.857	0.104	64.451	383.681	21.895
F-Value	36.782	3.479	471.250	295.914	11.958
Prob	<0.0001***	0.036*	<0.0001***	<0.0001***	<0.0001***

*Mean values with the same superscript are not significantly different; *p≤0.05; ***p≤0.001; n=3*

level of *Khoa* and *Paneer*, were 35.07-37.50 % (Singh et al. 2019), which is slightly lower as compared to moisture content of studied market *Gulabjamun*. The percent absorbed sugar in *Gulabjamun* of market samples were found statistically highly significant (p<0.001) with sample type (Table 2) and their values (Figure 1) ranged between 12.24±0.25 % (M1) to 37.65±0.52% (M3). The percent absorbed sugar of M1, M4 and M5 were statistically at par with each other whereas M2, M3 and M6 were not statistically at par with each other. The fat, moisture and percent absorbed sugar were found maximum for M6, M3 and M1, respectively whereas minimum for M2, M6 and M3, respectively (Table 1, Figure 1).

The GMD of Market *Gulabjamun* samples were found statistically highly significant (p<0.001) with sample type (Table 2) and the values (Figure 2) varied from 3.09±0.01 cm (M1) to 3.60±0.06 cm (M6). The GMD of samples M1, M3, M4 and M5 were statistically at par with each other whereas that of M2 and M6 was statistically at par with each other (Figure 2). The variation in diameter of market *Rasogolla* was 2.7±0.0 to 3.9±0.1cm (Srinivasa et al. 2017), which was similar to the GMD of market *Gulabjamun*. The sphericity of market *Gulabjamun* samples were found statistically highly significant (p<0.001) with sample type (Table 2) and it varied (Figure 3) from 0.94 (M1) to 0.97 (M6). The sphericity of market *Gulabjamun* samples M1, M2, M3 and M4 were statistically at par with each other whereas M1 was statistically different with M5 and M6 samples (Figure 3). The sphericity of *Gulabjamun* may depend on frying temperature of the balls and at lower temperature; balls obtain soft crust which may not retain its shape (spherical). The sphericity of any food near unity (1.00) may be considered as a spherical product for modelling of heat and transfer processes occurring during product production. The apparent density of studied market *Gulabjamun* samples ranged from 1.40±0.05 g/cm^3 (1 g/cm^3 = 1000 kg/m^3) to 1.84±0.32 g/cm^3 i.e. M5 (minimum) to M2 (maximum), respectively (Table 1). It was statistically significant (p≤0.05) with sample type (Table 1). Franklin et al. (2013) found that the average apparent densities of conventionally fried *Gulabjamun* at different temperatures i.e. 125, 135 and 145°C were 0.827, 0.808 and 0.775 g/cm^3, respectively. The unit-weight of market *Gulabjamun* samples were found

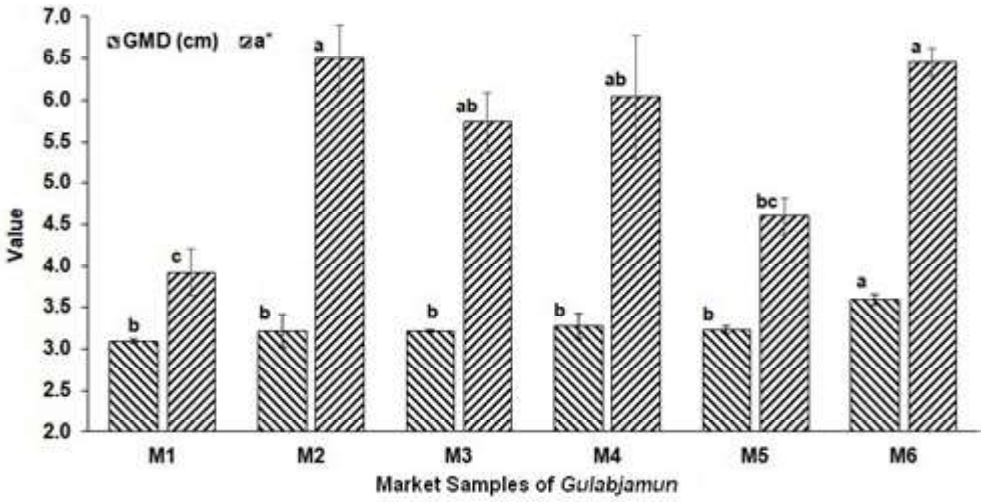

Fig. 2 Geometric mean diameter (GMD) and *a** values of Market *Gulabjamun*

Table 2 ANOVA for some physical and colour characteristics of Market *Gulabjamun*

Market *Gulabjamun*	Moisture (%)	Percentage Absorbed sugar (%)	GMD (cm)	Sphericity (fraction)	*L**	*a**
DF	5	5	5	5	5	5
SS	105.188	1454.819	0.440	0.002	290.118	16.630
MS	21.040	290.964	0.088	0.000	58.024	3.326
F-Value	96.016	1016.290	7.328	4.498	282.659	20.137
Prob	<0.0001***	<0.0001***	<0.01**	0.015*	<0.0001***	<0.0001***

*Mean values with the same superscript are not significantly different; * p≤0.05; ** p≤0.01; *** p≤0.001; n=3*

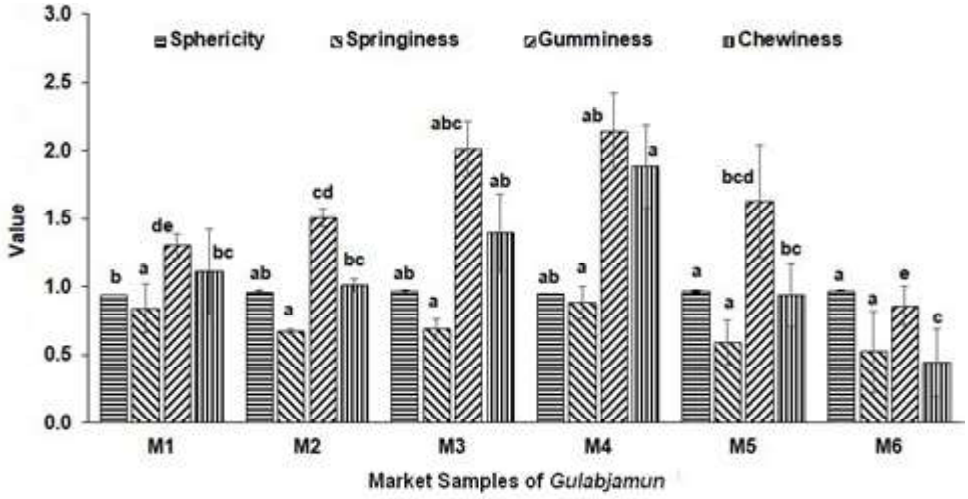

Fig. 3 Sphericity, springiness, gumminess, and chewiness of Market *Gulabjamun*

statistically highly significant (p≤0.01) with sample type (Table 3) and their values (Figure 1) varied from 24.21±0.29 g (M1) to 44.40±0.45 g (M6). The unit-weight of *Gulabjamun* sample M1 and M5 were found statistically at par with each other whereas that of M2 and M4 was statistically at par with each other (Figure 1). The samples M3 and M6 were statistically different with each other.

Colour characteristics of market *Gulabjamun*

The colour parameters of market samples *Gulabjamun* were studied using image analysis. Colour characteristics i.e. *L** and *a** (Table 2), *b** and browning index (Table 1) of studied market *Gulabjamun* samples were found statistically highly significant (p<0.001) with sample type (Table 3). The values of *L** (Figure 1) ranged (minimum to maximum) between 34.70±0.41 (M1) to 46.46±0.55 (M3). The *a** values ranged (minimum to maximum) between 3.92±0.28 (M1) to 6.50±0.39 (M2). The values of *b** and

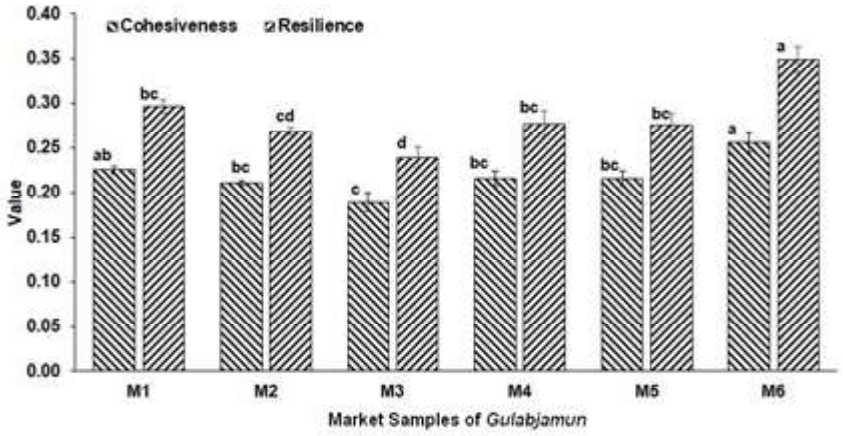

Fig. 4 Cohesiveness and resilience of Market *Gulabjamun*

Table 3 ANOVA for some textural characteristics and unit weight of Market *Gulabjamun*

Market *Gulabjamun*	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)	Resilience	Unit- weight (g)
DF	5	5	5	5	5	5
SS	0.292	0.007	3.308	3.497	0.20	1242.454
MS	0.058	0.001	0.662	0.699	0.004	207.076
F-Value	2.070	24.407	12.260	10.700	29.584	330.459
Prob	0.140^{NS}	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.01**

*Mean values with the same superscript are not significantly different; NS=non-significant; ** p≤0.01;*** p≤0.001; n=3*

browning index ranged (minimum to maximum) between -2.30±0.59 (M5) to 11.48±0.48 (M3), and 2.77±1.33 (M5) to 36.98±0.56 (M3), respectively (Table 1). The *L** values were found statistically at par with each other (Figure 1) for market *Gulabjamun* samples (i) M2 and M4 (ii) M5 and M6, and (iii) M1 and M6. The M3 sample showed maximum *L** value. It was reported that lightness value (*L**) of *Gulabjamun* Balls depends upon frying temperature of *Gulabjamun* Balls (deep fat frying). Lightness value (*L**) of the *Gulabjamun* Balls (deep fat frying) was ranged from 23.48 to 79.86 which was decreased gradually with increasing frying time (Kumar et al. 2006). In case of *a** values of market *Gulabjamun* samples, the M2, M3, M4 and M6 were found statically at par with each other whereas samples M1 and M5 also found statically at par with each other (Figure 2). The *b** values and browning index were found statistically at par with each other for (i) M1 and M6, and (ii) M2 and M4 (Table 1) in market *Gulabjamun* samples.

Textural characteristics of market *Gulabjamun*

The hardness, cohesiveness, gumminess, chewiness and resilience were found statistically highly significant ($p<0.001$) whereas springiness was found statistically non-significant ($p\geq0.05$) with sample type (Tables 1 and 3). The values for hardness (Table 1) ranged (minimum to maximum) between 3.36±0.56 N (M6) to 10.71±1.66 N (M3). The values for springiness, gumminess and chewiness ranged (minimum to maximum) between, 0.52±0.29 (M6) to 0.88±0.12 (M4), 0.86±0.13 N (M6) to 2.14±0.28 N (M4) and 0.44±0.24 N (M6) to 1.88±0.30 N (M4), respectively (Figure 3). The values for cohesiveness and resilience ranged (minimum to maximum) between, 0.18±0.01 (M3) to 0.25±0.01 (M6), and 0.24±0.01 (M3) to 0.35±0.02 (M6), respectively (Figure 4). The hardness was found statistically at par with each other for market *Gulabjamun* samples (i) M3, M4 and M5 (ii) M1, M2, and M5, and (iii) M1 and M6 (Table 1). In market *Gulabjamun* samples, the cohesiveness and resilience were found statistically at par with each other for (i) M1, M2, M4, and M5, and (ii) M2 and M3 (Figure 4). For market *Gulabjamun* samples, the gumminess was found statistically at par with each other for (i) M2, M3, M4, and M5 (ii) M1, M2 and M5, and (iii) M1 and M6 (Figure 3). The chewiness was observed statistically at par with each other for market *Gulabjamun* samples for (i) M1, M2, M3, and M5 (ii) (i) M1, M2, M5, and M6, and (i) M3 and M4 (Figure 3). Aneja *et al*. (2002) reported that the hardness (N), springiness (mm), cohesiveness, gumminess (N), chewiness (N.mm) of *Gulabjamun* as 14.09 N, 7.88 mm, 0.123, 1.75 N, and 14.21 N.mm, respectively. So, the hardness, springiness, cohesiveness and chewiness of *Gulabjamun* were found less than market sample of *Gulabjamun*.

Therefore, there was statistically significant difference was observed in the characteristics of selected brands of market *Gulabjamun* samples which may be due to the variations in

various steps at processing, packaging, storage and distribution stages of studied market *Gulabjamun* samples.

Conclusions

The various quality characteristics i.e. physico-chemical, colour and textural characteristics of six brands of *Gulabjamun* were determined and statistical analysis was carried out. The fat and unit-weight of market *Gulabjamun* were found statistically highly significantly different . The moisture content, percent absorbed sugar, GMD, sphericity of studied market *Gulabjamun* samples were found statistically highly significant. Apparent density was statistically significant. The hardness, cohesiveness, gumminess, chewiness and resilience were found statistically highly significant whereas springiness was found statistically non-significant ($p>0.05$). The size of market *Gulabjamun* (3.09±0.01 to 3.60±0.06 cm) may be taken as 3.0-3.6 cm for development of process equipments for *Gulabjamun* production.

Acknowledgement

The authors acknowledge fellowship and infrastructure received from ICAR-NDRI, Karnal to carry out the research work.

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Indian J Dairy Sci 74(6): 486-491
https://doi.org/10.33785/IJDS.2021.v74i06.003

RESEARCH ARTICLE

Optimization of fat content to develop goat milk *shrikhand*

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Received: 14 July 2021 / Accepted: 03 October 2021 / Published online: 21 December 2021

Abstract: The present study was conducted to optimize the fat content of goat milk shrikhand using goat milk with three different fat % *viz*. 4.0 (F1), 5.0 (F2) and 6.0 (F3) %. The pH values decreased while titratable acidity increased significantly (P<0.05) with increased fat content. Fat content and brix values increased significantly (P<0.05) with increased fat content in goat milk. All textural parameters *i.e*. firmness, consistency, cohesiveness and work of cohesiveness increased significantly (P<0.05) with increased fat content. Among the sensory attributes, there was no significant difference in color and appearance, texture and mouth coating scores among the treatments; however flavor, sweetness and overall acceptability scores increased significantly (P<0.05) with increased fat content. There was no significant difference between F2 and F3 for any sensory attribute including overall acceptability. Present day health conscious consumer demand product with lower fat content without compromising with taste and flavour. Therefore, F2- goat milk shrikhand prepared with 5.0% fat was found optimum and selected as the best treatment.

Keywords: Goat milk shrikhand, Fat content, Optimization, Textural and colour parameter, Sensory evaluation.

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Introduction

India is leading milk producer in the world with 187.7 million tonnes of milk (NDDB, 2019) due to advancement of technology, proper nutrition and appropriate managemental practices. Livestock contributes about 9.2% in gross value added (GVA) and 26.2 % in agriculture sector in India. The livestock population in India includes 302.3 million bovines, 74.3 million sheep, 148.9 million goats, about 9.1 million pigs and 851.8 million poultry. The rural and urban population of goat is 129.081 million and 6.092 million respectively in India. Total goat milk production in India is 6.09 million tones, out of which Rajasthan and Uttar Pradesh produce 2.31 million tonnes and 1.34 million tonnes respectively (DAHD, 2019). Goat milk production is a dynamic and growing industry that is fundamental to the wellbeing of millions people worldwide and is an important part of the economy in India. Goat milk is having better digestibility, alkalinity, buffering capacity and certain therapeautic values in medicine and human nutrition (Park and Chukwu, 1989; Park, 1994) in comparison to cow's or human milk. The goat milk microbiota is also considered a good source of novel bacteriogenic *Lactic acid* bacteria (LAB) strains that can be exploited as an alternative for use as bio preservative in food (Perin and Nero, 2014). It is also rich source of amino acid, being 20-40 folds higher than cow milk (Mehaia and Al-Kanhal, 1992) which is involved in bile salt formation, osmoregulation, antioxidation, calcium transport and in the central nervous system (Redmond et al. 1998). Minerals content such as calcium, potassium, magnesium and chloride as well as vitamin A, B, C, D, thiamin and niacin content of goat milk is higher than that of cow milk (Chandan et al. 1992). Goat milk also contains higher content of three characteristics fatty acids *i.e.* caproic acid, caprylic and capric acid which are having medicinal values for patients suffering from malabsorption, childhood epilepsy, cystic fibrosis and gallstones (Haenlin, 1992); however these are responsible for intense "goaty flavour" which limits the acceptability of goat milk products among the consumers (Ozer et al. 2017).

Traditional dairy foods have always played a pivotal role in preservation of precious milk nutrients and promotion of its consumption among masses. Shrikhand is one of the widely

relished indigenous milk product prepared by the fermentation of milk by using known strain of lactic acid bacteria. It is very much popular in western part of India due to its high nutritive, characteristics flavour, taste, palatable nature and possible therapeutic value. Shrikhand contains appreciable amount of milk protein and phospholipids and is obtained by lactic acid fermentation through the action of starter culture consisting *Lactobacillus bulgaricus, Streptococcus lactis, Streptococcus diacetylactis, Lactobacillus citrovoroum and Streptococcus thermophilus.* It is produced from chakka which in turn is obtained from dahi (curd) after draining off the whey. The basic ingredients sugar, colour and flavour are thoroughly mixed into chakka and to form a sort homogenous mass called Shrikhand (Desai and Gupta, 1986). The popularity of fermented dairy products from goats' milk has shown a gradual increase all over the world due to its better functional properties and health benefits. As per FSSAI (2016), shrikhand should contain 58% total solids (min.), 8.5 % fat (min.) 9.0% protein (min.), 1.4% titratable acidity (max.) and0.9% ash (maximum). Fat is a very important ingredient contributing to the texture, flavor and overall perception of dairy products. Milk fat has a complex and rich chemical composition and provides unique sensorial properties (flavor and mouthfeel) to milk and milk products.

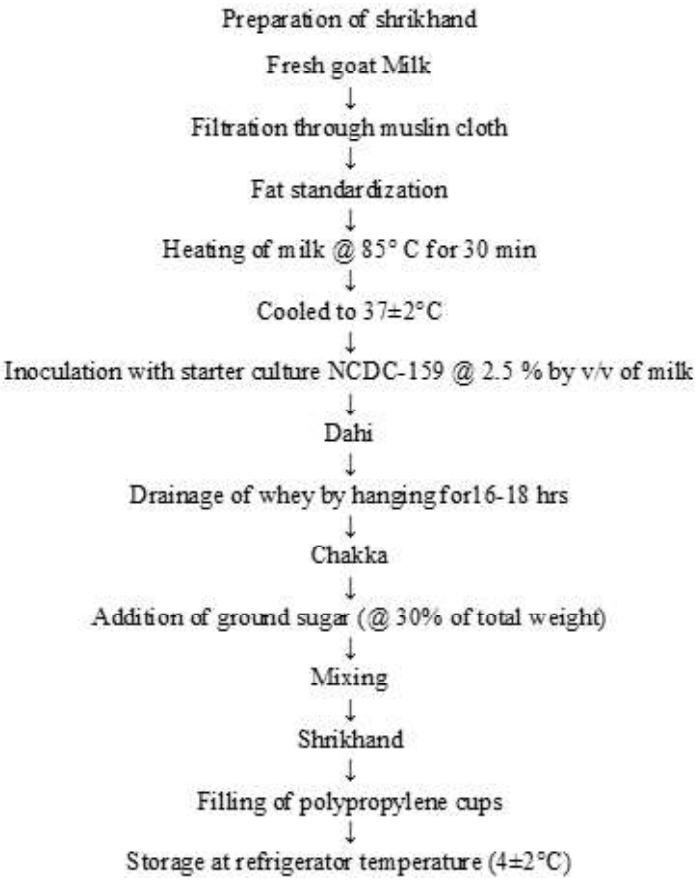
Consumers are becoming increasingly conscious nowadays about their nutrition, hygiene and wellbeing. Consumption of more fat and calories has been related to the coronary hear diseases, and obesity (Law et al. 1991). There is evidence tha high fat intake is associated with increased risk of obesity, cancer high blood cholesterol and coronary heart diseases (Garcia et al 2002). The number of patients with cardiovascular diseases i growing rapidly in India, so there is a dire need to develo functional products with low fat content (Anand et al. 2015) Present day health conscious consumer demand a healthy produc with less fat content, again dairy products from goat milk ha less acceptability in terms of flavour and aroma due to presenc of medium chain fatty acids. Therefore, development of shrikhan with low fat goat milk may be a technological challenge wit higher organoleptic and nutritional quality. Keeping these view in mind, present study was carried out to optimize the fat conten of goat milk shrikhand with well acceptability in terms of nutrien content and sensory evaluation.

Materials and Methods

The experiments were carried out in the Department of Livestoc Products Technology, College of Veterinary Sciences and Anima Husbandry, U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigya Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura 281001 (UP), India. Fresh clean wholesome milk of goat wa procured from Department of Veterinary Physiology, DUVASU Mathura. Starter culture (NCDC-159) was procured from NDRI Karnal which contained mixed culture of *Lactococcus lactis Lactococccus diacetylactis* and *Lactococcus cremoris.* Th culture was activated to as per the standard method and the activated parent culture was maintained by sub culturing and stored under refrigeration. Clean crystalline sugar was procured from local market of Mathura. All the chemicals used in the study were of analytical grade and procured from Hi Media laboratories (P) Ltd, Mumbai.

Preparation of Shrikhand

The shrikhand was prepared as per method described by Gupta et al. (2018) with slight modifications. Fresh goat milk was filtered through muslin cloth and then fat content was standardized using Pearson square method, where skimmed milk powder or cream was added to adjust fat content in milk. Then milk was subjected to heat treatment at 85 °C for 30 minutes followed by cooling at 37±2°C. Milk was inoculated with NCDC-159 @ 2.5 % by v/v of milk and incubated at 35-37 °C for 12-15 hours for proper curd setting. The curd thus obtained was transferred to clean muslin cloth and hanged for 16-18 hours in order to drain the whey to obtain chakka. The chakka was kneaded to have uniform consistency and then mixed with 30% ground sugar. Finally shrikhand was filled in pre sterilized thermorigid polypropylene cups and stored at under refrigeration at 4±2°C. In present study, following abbreviations were used for present experiment: F1- goat milk shrikhand prepared with 4.0% fat, F2- goat milk



shrikhand prepared with 5.0% fat and F3- goat milk shrikhand prepared with 6.0% fat.

Analytical methods

Physic-chemical properties

The pH of shrikhand was determined by using digital pH meter (WTW, Germany, model pH 330i) as per method given by Trout et al. (1992). Water activity of each sample was measured three times in duplicate using a water activity meter (AquaLab 3 TE, Inc. Pullman, WA) at Department of Goat Products Technology, CIRG, Makdhoom. Proximate parameters viz. moisture, protein, fat and ash content were estimated as per AOAC (1995).

Textural and colour parameters

The texture profile analysis of shrikhand was done with the help of instrumental texture profile analyser (TA HD Plus Texture analyser) for firmness, consistency, cohesiveness and work of cohesiveness (Bourne, 1978). Texture analyzer equipped with 5 kg load cell and back extrusion test using 35 mm cylinder probe was used for texture profile analysis of the samples. Other conditions (test descriptions) set for analyses were as follows:

Mode:	Measure force in compression
option:	Return to start
Pre-test speed:	1 mm/sec
Test speed :	1mm/sec
Post-test speed :	10mm/sec
Distance :	30mm
Trigger type:	Auto (F) -10g
Trigger force:	0.04903 N
Tare mode:	Auto
Data acquisition rate :	400pps
Probe:	Back extrusion cell (A/BE)

The colour parameters *i.e.* lightness (L^*), redness (a^*) and yellowness (b^*) of the shrikhand were measured using Hunter colourimeter of ColourTech PCM+ (Colour Tec Associates Inc. Clinton NJ, USA) at Department of Goat Products Technology, CIRG, Makdhoom.

Sensory evaluation

Sensory evaluation was conducted by experienced semi trained panellists using 8-point descriptive scale (where 1= extremely disliked and 8= extremely liked) (Keeton, 1983) for colour and appearance, flavour, texture, sweetness, mouth coating and overall acceptability.

Statistical analysis

The data obtained in the study on various parameters were statistically analyzed on 'SPSS-16.0' software package as per standard methods of Snedecor and Cochran (1995). Duplicate samples were drawn for each parameter and the experiment was replicated thrice (n=6). Sensory evaluation was performed by a panel of seven member judges three times, so total observations being 21 (n=21) Data were subjected to one way analysis of variance, homogeneity test and Duncan's Multiple Range Test (DMRT) for comparing the means to find the effects between samples.

Results and Discussion

Composition of goat milk

Total 30 goat milk samples were evaluated for proximate composition and reported to have following composition on an average as per table 1.

Ibrahim et al. (2020) also reported the average composition of fresh goat milk as 3.54% protein, 3.25% lactose, 6.68% fat and 6.82% SNF respectively. These differences in fat content might be due to animal type, oldness, competition, season, location, and feedstuffs (Contreras et al. 2015). Santis et al. (2019) developed goat milk yogurt with probiotic culture containing *Streptococcus thermophilus, Lactobacillus delbrueckii spp. bulgaricus*, with cultures of *Leuconostoc lactis* and fresh raw milk was standardized at 3.74 % fat, p3.47%rotein, 3.92%lactose and pH 6.91. Based on available literature, several preliminary trials were conducted to standardize the processing technology of goat milk shrikhand. The final formulation of goat milk shrikhand was optimized following the method prescribed by Gupta et al. (2018) with slight modifications as milk was standardized at lower fat content, again due to poor rheological properties and soft curd formation from goat milk, temperature time combination was changed as per the standard method. The curd was also hanged up for comparatively more time (16-18 hours) as compared to standard method.

Physico-chemical properties

The physico-chemical properties of goat milk shrikhand prepared with different fat content are presented in table 2. The pH values decreased while titratable acidity increased significantly ($P<0.05$) with increased fat content, however there was no significant difference in titratable acidity of F1 and F2. The pH of product in present study (4.24 to 4.57) is in agreement with the range of 4.15 to 4.50 given in the literature (Kavas et al. 2003; Matos et al. 2013). There was no significant difference in proximate parameters *i.e.* moisture, protein and ash content except fat content which

Table 1 Proximate composition of goat milk (n =30)

S. No.	Component	%
1	Fat	5.32
2	SNF	7.79
3	Lactose	4.3%
4	Protein	2.84

increased significantly (P<0.05) with increased fat content in goat milk. There was no significant difference in water activity among the treatments; however brix value increased significantly (P<0.05) with increased fat content. Higher brix values with increased fat content in shrikhand might be due to higher viscosity and compactness of product with increased fat content. Falke (2015) also observed significant (P<0.05) increase in brix values of yogurt with increased fat content and addition of inulin due to higher solid content.

Textural and colour parameters

The values of textural and colour parameters of goat milk shrikhand prepared with different fat content are presented in table 3. All textural parameters *i.e*. firmness, consistency, cohesiveness and work of cohesiveness increased significantly (P<0.05) with increased fat content; where no significant difference was observed between F2 and F3 in cohesiveness values. Higher textural parameters values with increased fat content might be due to more firm and smooth curd/chakka produced by milk containing higher fat percentage. Somayeh et al. (2017) also reported higher hardness and consistency values of yogurt with increased fat content. Amatayakul et al. (2006) and Soukoulis et al. (2007) also reported that the change in fat content of milk might significantly (P<0.05) effect the firmness and other textural parameters of fermented milk products. There was no significant difference in lightness, redness and yellowness values among the treatments due to no effect of fat content variation in colour of milk and milk products. Chudy et al. (2020) reported that natural colour of milk is due to the reflection of light by dispersed fat globules, calcium caseinate and calcium phosphate.

Sensory evaluation

The sensory scores of goat milk shrikhand prepared with different fat content are presented in table 4. Fat has an important role in the development of flavor, texture and appearance of milk products (Sipahioglu et al. 1999). There was no significant difference in color and appearance, texture and mouth coating scores among the treatments; however flavor, sweetness and overall acceptability scores increased significantly (P<0.05) with increased fat content. F2 had significantly (P<0.05) higher flavor scores than F1; however scores of F3 were comparable to F1 and F2. F3 had slightly lower flavour scores than F2 inspite of higher fat content due to pronounced goaty flavour at higher fat content in milk. As per Park et al. (2007), caprylic, capric, and caproic acid present in goat milk might lower the acceptability of goat milk products due to goaty aroma and flavour. Singh et al. (2015)

Table 2 Physio-chemical properties (Mean±SE) of goat milk shrikhand prepared with different fat content

Parameters	F1	F2	F3	Treatment Mean
pH	4.57^{a}±0.03	4.40^{b}±0.01	4.24^{c}±0.02	4.40±0.03
Titratable acidity	0.41^{b}±0.05	0.42^{b}±0.01	0.46^{a}±0.01	0.43±0.01
Moisture (%)	47.43±0.51	47.18±0.39	46.96±0.39	47.19±0.24
Protein (%)	6.55±0.06	6.52±0.05	6.51±0.09	6.52±0.03
Fat (%)	10.25^{c}±0.11	13.96^{b}±0.20	15.43^{a}±0.26	13.21±0.54
Ash (%)	0.64±0.02	0.65±0.01	0.66±0.01	0.65±0.01
Water activity	0.948±0.07	0.941±0.26	0.938±0.13	0.942±0.10
Brix value	27.82^{c}±0.02	31.29^{b}±0.02	33.46^{a}±0.04	30.85±0.02

F1- goat milk shrikhand prepared with 4.0% fat
F2- goat milk shrikhand prepared with 5.0% fat
F3- goat milk shrikhand prepared with 6.0% fat
n=6

Table 3 Textural and colour parameters (Mean±SE) of goat milk shrikhand prepared with different fat content

Parameters	F1	F2	F3	Treatment Mean
Firmness	66.74^{c}±0.32	67.91^{b}±0.16	69.00^{a}±0.19	67.55±0.35
Consistency	43.36^{c}±0.13	44.30^{b}±0.11	45.97^{a}±0.17	44.54±0.27
Cohesiveness	32.24^{b}±0.31	33.70^{a}±0.12	33.98^{a}±0.12	33.31±0.19
Work of cohesiveness	25.37^{c}±0.18	26.54^{b}±0.14	27.36^{a}±0.11	26.42±0.21
Lightness *(L*)*	76.23±0.02	77.92±0.01	77.89±0.02	77.34±0.01
Redness *(a*)*	4.42±0.11	4.52±0.08	4.48±0.09	4.47±0.05
Yellowness *(b*)*	7.25±0.10	7.21±0.09	7.11±0.11	7.19±0.05

F1- goat milk shrikhand prepared with 4.0% fat
F2- goat milk shrikhand prepared with 5.0% fat
F3- goat milk shrikhand prepared with 6.0% fat
n=6

Table 4 Sensory evaluation (Mean±SE) of goat milk shrikhand prepared with different fat content

Attributes	F1	F2	F3	Treatment Mean
Colour and appearance	6.81±0.04	6.90±0.14	6.92±0.04	6.88±0.05
Flavour	6.55^{b}±0.09	6.87^{a}±0.07	6.75^{ab}±0.08	6.72±0.04
Texture	6.69±0.13	6.77±0.08	6.68±0.08	6.71±0.05
Sweetness	6.67^{b}±0.09	6.98^{a}±0.07	7.01^{a}±0.09	6.88±0.05
Mouth coating	6.53±0.07	6.87±0.07	6.89±0.08	6.76±0.04
Overall acceptability	6.79^{b}±0.07	6.99^{a}±0.05	7.01^{a}±0.04	6.93±0.03

F1- goat milk shrikhand prepared with 4.0% fat
F2- goat milk shrikhand prepared with 5.0% fat
F3- goat milk shrikhand prepared with 6.0% fat
n=21

developed shrikhand with different levels of fat and sugar and observed that shrikhand prepared with 6% fat and 40% sugar had highest overall acceptability among the treatments. Shelke et al. (2014) also reported that well acceptable low fat low sugar mango flavoured shrikhand was prepared with 70% skimmed milk based chakka and 40% sugar. Sweetness and overall acceptability scores of F2 and F3 were significantly ($P<0.05$) higher than F1 in present study; however there was no significant difference between F2 and F3.Present day health conscious consumer demands for low fat products without compromising the organoleptic qualities. Therefore, keeping the view of health concern, F2- goat milk shrikhand prepared with 5.0% fat was found optimum in terms of nutrient content and sensory properties.

Conclusions

People all over the world are more heath conscious and choosing healthy diet options to maintain a healthy lifestyle. Increasing awareness about health benefits related to low-fat dairy products among the global population is expected to propel its demand across the globe. The present study was conducted to optimize the fat content of goat milk shrikhand which was a challenge due to lower fat content and total solid content in goat milk alongwith poor textural properties and soft curd formation. However, increasing fat content in goat milk had significant effect on textural and colour parameters. Fat content also improved sensory qualities of product in terms of flavour, sweetness and overall acceptability scores. Goat milk shrikhand prepared with 5.0% fat was selected as the best treatment. Goat milk products have lower acceptability among people inspite of their medicinal properties and easy digestibility due to presence of medium chain fatty acids like capric, caprylic acids, therefore further research may be recommended for incorporation of fruit pulp and other natural flavoruing compounds in goat milk shrikhand to improve it's acceptability.

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Indian J Dairy Sci 74(6): 492-497
https://doi.org/10.33785/IJDS.2021.v74i06.004

RESEARCH ARTICLE

Effect of red plum on quality characteristics of banana milk smoothies

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Received: 20 April 2021 / Accepted: 31 August 2021 / Published online: 21 December 2021

Abstract : The present study was carried out to enhance the functional quality of milk smoothies. Experiments were conducted on various formulations of smoothies using banana (T1 without plum) and red plum in different proportions (75:25 (T2), 50:50 (T3), and 25:75 (T4)) along with Sahiwal cows milk. Results revealed that physico-chemical quality characteristics of control (T1 banana-based smoothie) and smoothie replaced with red plum differ significantly ($P<0.05$) for moisture, protein, fat, fiber, sugar, total solid, solid-not fat, pH, and vitamin-C. Smoothie prepared with 50 % banana and 50 % red plum (T3) rated higher sensory scores for all sensory attributes than other groups. Among all milk smoothies, T3 group was selected best on the basis of nutritional, physicochemical, and sensory qualities.

Keywords: Banana, Milk smoothie, Physico-chemical quality, Red plum, Sensory profile

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Introduction

Appearance of the smoothie is like milk sake, semi solid in consistency. Smoothie is dairy products commonly prepared with fruit and milk and provides health promoted compounds (minerals, vitamins, fiber, ploy phenolics) to the consumers. Milk and milk products are rich in good quality amino acids and fatty acids. However, in general milk is deficient in fiber and vitamin-C. India ranks first in milk production and produced 187.7 million metric tons (DAHD & F) in the 2018-19. The red plum is good source of vitamin-C, fibre and polyphenolic compounds etc. Consumers are very health conscious nowadays therefore; they demand healthy food products in terms of nutritive value and palatability. The nutritional quality of foods basically milk and fruit based products depends not only on the nutrient content of fruits but also on the processing conditions (Kumar et al. 2018). Addition of fresh fruits and/or vegetables in milk products improves its nutritional quality, sensorial attributes and also increased its functionality. Smoothie prepared with the mixing of fruits and milk also improves its minerals and vitamin content in milk products as well increases the absorption capacity of other nutrients.

Red Plum (*Prunus domestica*) is a fruit that belongs to the family of Rosaceae subgenus Prunus of the genus Prunus. Hassan et al. (2015) reported that phytoactive compounds present in plums have capacity to minimize the harmful effects caused by free radicals and protect against various life style diseases. Vitamin-C and various phytoactive compounds are an excellent anti-oxidant compounds present in plum can also prevent oxidative deterioration caused by free radicals in foods and inside human body. Plum is also a good source of fiber and antioxidants, which improves digestion and metabolism. Eating of plums boosts bone health, especially post-menopausal women Nourbakshi et al. (2013). So the present investigation was carried out to study the technology development and physico-chemical, nutritional and sensory assessment of fruits and milk based milk smoothies.

Materials and Methods

Sahiwal cow milk collected from the university dairy farm. Red plum, banana, sugar procured from the local market Mathura.

Analytical reagents used in this study were purchased from the local supplier.

Method of preparation of banana and red plum milk smoothies

Clean and pasteurized milk of Sahiwal cow was used for preparation of milk smoothies. Smoothies were prepared in four separate groups i.e. control using banana T1 (without red plum) and treated groups added plum in different proportions T2, T3, and T4 using three different proportions of banana and red plum (75:25, 50:50 and 25:75) in each formulation. The two percent sugar was added in all variants of milk smoothies. At last sodium alginate @ 0.1 percent was added in formulation to make the desired consistency. The smoothies were developed following the procedure as described in flow diagram Fig. 1.

Analytical procedure

Proximate and physico-chemical quality characteristics of milk smoothies

Moisture, protein, fat, fiber, ash, total solid and solid not fat in milk smoothies were determined as per method described by AOAC (2000).

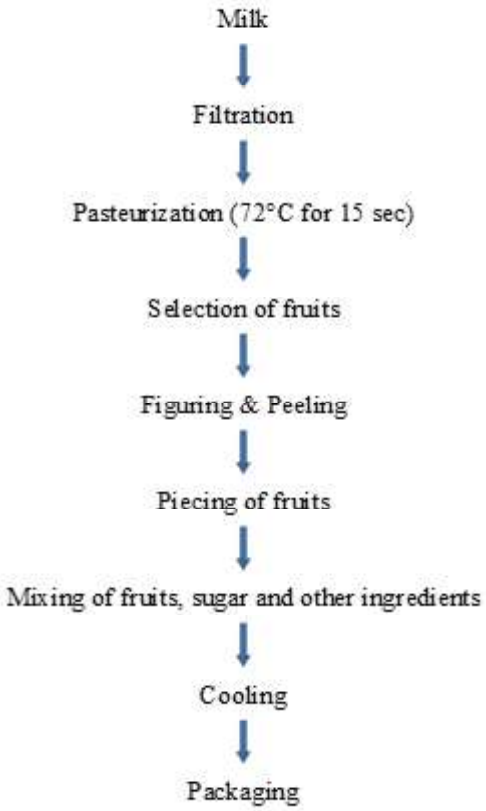

Fig. 1 Flow diagram for preparation of milk smoothies

Sugar

The sugar content in milk smoothies was estimated using the procedure of Pandya et al. (2013). In this procedure milk products were first made protein and fat free by adding 23 ml of HCl to 70 ml of milk sample. HCl deproteinizes the milk and all the proteins coagulated and settled in the bottom of the flask. The supernatant was separated. Its quantity was found out to be 23ml. It was further diluted with 77 ml milk to make the volume up to 100 ml. Then samples were heated with alkaline copper solution by using a special type of Folin-Wu tubes. Glucose and other reducing substances reduce copper from the cupric ions to the cuprous ions. When phosphomolybdic acid was added it was reduced from cuprous form to blue coloured molybdenum blue. Intensity of molybdenum blue is directly proportional to sugar. Its concentration was measured calorimetrically with a known standard at 420 nm.

pH

pH was determined by using digital pH meter (WTW, Germany, model pH 330i) by immersing the spear type combination electrode (Sentix®, Germany) directly into milk sample. The pH meter was calibrated prior to measurement every time as per the manufacturer's instructions using known buffers of pH 7.0 and 4.01.

Titratable Acidy

The measurement of titratable acidity in milk smoothies was according to the method described by Caric et al. (2000). Erlenmeyer flask used with a transfer pipette 20 ml smoothie and 1 ml of 2% w/v solution of phenolphthalein. Content was titrated with 0.1 M NaOH solution for appearance of faint pink color that will not get lost for over 2 minutes. Acidification of smoothie is calculated by the formula: $K = V^{*} \cdot 2$, where V- volume is consumed base neutralization.

Specific gravity

The specific gravity was determined according to the method of AOAC (2000) with some modifications. The density of milk smoothie was measured against the density of standard (water). Firstly, pre-weighed vessel was filled with standard reference fluid (water) to some predetermined level at 20°C and weight was taken. Secondly, milk smoothie was filled in similar vessel at similar level and temperature and weighed. Specific gravity of milk smoothie was calculated by the following formula:

$$\text{Specific gravity} = \frac{\text{Weight of milk sample}}{\text{Weight of distilled water}}$$

Vitamin-C

Vitamin-C in milk smoothies were estimated using the procedure of Hassan et al. (2016). In which 10 gm of milk smoothie was transferred into 100 ml volumetric flask homogenized by using 50 ml acetic acid solution with shaken, 4-5 drops of bromine water has been added until the solution became colored. Then a few drops of thiourea solution were added to it to remove the excess bromine and thus the clear solution was obtained. Then 2, 4-Dinitrophenyl hydrazine solution was added thoroughly with all standards and also with the oxidized ascorbic acid. Then complete the solution up to the mark with acetic acid. The absorbance for all samples was measured using spectrophotometer to determine the concentration of ascorbic acid in the smoothies.

Sensory evaluation

The sensory quality of milk smoothies were evaluated using 8 point descriptive scale (Keeton et al. 1983) where 8 denoted extremely desirable and 1 denoted extremely poor. A sensory panel (semi trained) of seven judges selected from post-graduate students and faculty of Veterinary College, DUVASU, Mathura were examined the various sensory attributes viz., color and appearance, flavour, body and texture, sweetness, acidity, consistency, mouthfeel and overall acceptability.

Statistical analysis

Data were analyzed using the software, Statistical Package for Social Sciences (SPSS) at the 0.05 level following the procedure of Snedecor and Cochran (1994). Duplicate samples were drawn for each parameter and the experiment was replicated thrice (n=6). Sensory evaluation was performed by a panel of seven member judges three times, so total observations of recorded for each sensory attribute were 21 (n=21). Data were subjected to one way analysis of variance, homogeneity test and Duncan's Multiple Range Test (DMRT) for comparing the means to find the effects between treatments.

Results and Discussion

Proximate composition of milk smoothies

The percent means of moisture content in T1, T2, T3, and T4 were 82.27±0.04, 82.39±0.05, 83.47±0.04, and 83.99±0.05 respectively Table 1. The highest mean value of moisture was in T4 while the lowest was in T1. The analysis of variance indicated significant ($P<0.05$) difference in mean moisture content among different treatments. Moisture content in treatment groups has a positive correlation with the replacement of banana with red plum. The variation in moisture content in the smoothie might be due to the variation in content of banana and red plum. It could be due to higher moisture in plum as suggested by (Munmun, 2005) during the preparation of jellies like products. Saranyambiga et al. (2017) also reported that increased moisture percent on the addition of Jamun fruit in milk smoothie than control. The percent mean values of fat in T1, T2, T3, and T4 were 2.27±0.04, 2.49±0.05, 2.53±0.01, and 2.79±0.05 respectively Table 1. The highest mean value of fat was in T4 while the lowest was in control. The analysis of variance indicated no significant ($P>0.05$) difference in the mean fat content of T2 and T3 while these values were significantly ($P<0.05$) different with the mean fat values of T1 and T4. Banana added milk smoothie has lower fat content than red plum added smoothie. The reason might be higher fat in red plum (2 percent) as compared to banana (0.33 percent). The same findings were given by Akther et al. (2012) during the preparation of plum based leather or jelly-like products. The percent mean contents of protein was comparatively higher in banana milk (2.83±0.02) smoothie than red plum added smoothie T2 (2.74±0.02), T3 (2.71±0.02), and T4 (2.69±0.05) respectively Table 1. Protein content in smoothies prepared with a higher amount of red plum showed a decreasing pattern with an increase in red plum which could be due to the lower amount of protein in plum than a banana. These findings are very well justified with the report of Balaswamy et al. (2013) on the development of smoothies from selected fruit pulps/juices and Akther et al. (2012) for plum based jelly products. The percent mean contents of ash in T1 (0.97±0.04), T2 (0.95±0.02), T3 (0.94±0.02), and T4 (0.91±0.01) respectively. The highest mean value of ash was in T1 while the lowest was in T4 Table 1. The analysis of variance indicated no significant ($P>0.05$) difference in mean ash content among different treatments. The ash content in all groups of milk smoothies remains the same which might be due to all most comparable mineral content in banana and red plum. The percent mean contents of fiber in T1, T2, T3, and T4 were 0.98±0.04, 1.05±0.02, 1.18±0.05, and 1.29 ±0.05 respectively Table 1. The highest mean value of fiber was in T4 while the lowest was in T1. The analysis of variance indicated a significant ($P<0.05$) increase in mean fiber values from T1 to T3 and T4. The higher fiber in higher plum based smoothies could be attributed to higher fiber

Table 1 Ingredients used in the preparation of milk smoothie

Ingredients	T1	T2	T3	T4
Milk (g)	125	125	125	125
Banana (g)	75	56.25	37.50	18.75
Red plum (g)	-	18.75	17.50	56.25
Sugar (%)	2/4/6	4	4	4
Sodium alginate (%)	0.1	0.1	0.1	0.1

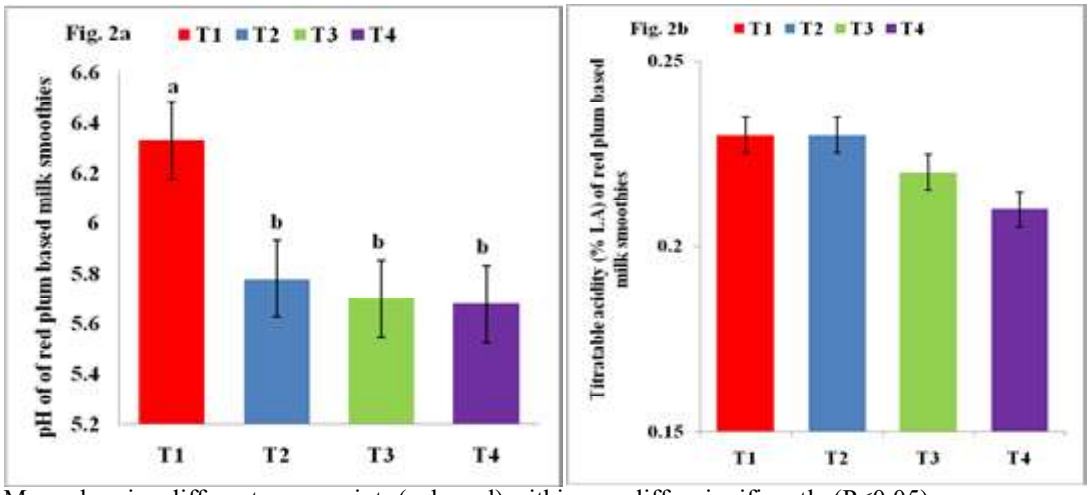

Fig. 2 pH and Titratable acidity (% LA) of banana and red plum based milk smoothies

Means bearing different superscripts (a, b, c, d) within row differ significantly (P<0.05)

Where: T1= Banana based milk smoothie

T2= 75% Banana and 25% red plum based milk smoothie

T3=50% Banana and 50% red plum based milk smoothie

T4=25% Banana and 75% red plum based milk smoothie

Table 2 Treatment wise physico-chemical characteristics of banana and red plum based milk smoothies (Means±SE)

Parameters	Banana and red plum based milk smoothies			
	T1	T2	T3	T4
Moisture (%)	82.27^{d}±0.04	82.39^{c}±0.05	83.47^{b}±0.04	83.99^{a}±0.05
Fat (%)	2.27^{c}±0.04	2.49^{b}±0.05	2.53^{b}±0.01	2.79^{a}±0.05
Protein (%)	2.83^{a}±0.02	2.74^{ab}±0.02	2.71^{b}±0.02	2.69^{b}±0.05
Ash (%)	0.97±0.04	0.95±0.02	0.94±0.02	0.91±0.01
Fibre (%)	0.98^{c}±0.04	1.05^{bc}±0.02	1.18^{ab}±0.05	1.29^{a}±0.05
Sugar (%)	10.68^{a}±0.03	10.38^{b}±0.02	9.17^{c}±0.03	8.33^{d}±0.17

- Means bearing different superscripts (a, b, c, d) within row differ significantly (P<0.05)
- Where: T1= Banana based milk smoothie
  T2= 75% Banana and 25% red plum based milk smoothie
  T3=50% Banana and 50% red plum based milk smoothie
  T4=25% Banana and 75% red plum based milk smoothie

contents in plum (4 percent) than a banana (2.6 percent) as suggested by Walkling-Ribeiro et al. (2010) during shelf life assessment of fruit-based smoothies. The percent mean contents of sugar in T1, T2, T3, and T4 were 10.68±0.03, 10.38±0.02, 9.17±0.03, and 8.33±0.17 respectively Table 1. The highest mean value of sugar was in the milk smoothie T1 while the lowest was in T4. The analysis of variance indicated a significant (P<0.05) decrease in mean sugar content of different treatments with increased levels of red plum in the formulation. Contrary to these findings, contents of sugar, total solids, and solid-not-fat showed a decreasing pattern due to lower amounts of minerals and other substances in plum than banana as suggested by Keenan et al. (2011) during the quality assessment of fruit-based smoothies.

Physico-chemical quality of milk smoothie

The percent mean contents of total solids were in T1 (17.73±0.02), T2 (17.61±0.01), T3 (16.53±0.01), and T4 (16.01±0.01) respectively Table 2. The highest mean value of total solids was in T1 while the lowest was in T4. The analysis of variance indicated a significant (P<0.05) difference in mean total solids content among T1, T3, and T4. The percent mean contents of Solids-not-fat in T1, T2, T3, and T4 were 15.46±0.03, 15.12±0.01, 14.00±0.11, and 13.22±0.01 respectively Table 2. The highest mean value of SNF was in T2 while the lowest was in T4. The analysis of variance indicated a significant (P<0.05) difference in mean SNF content among T1, T3, and T4. However, some contrary reports were also noticed on values of sugar and Solid-not-fat which might be due to the overall effect of banana and red plum on the composition of smoothies as reported by Rao et al. (2002) in milk products prepared with blending of orange juice in skim milk powder. The

Table 3 Physico-chemical quality of red plum based milk smoothies (Means±SE)

Physico-chemical quality	Banana and red plum based milk smoothies			
	T1	T2	T3	T4
Total Solids (%)	17.73^{a}±0.02	17.61^{ab}±0.01	16.53^{c}±0.01	16.01^{d}±0.01
Solids-not-fat (%)	15.46^{a}±0.03	15.12^{ab}±0.01	14.00^{c}±0.11	13.22^{d}±0.01
Specific gravity	1.061±0.01	1.066±0.02	1.069±0.01	1.071±0.01
Viamin-C (mg/100g)	3.55^{d}±0.03	4.98^{c}±0.05	6.29^{b}±0.05	7.61^{a}±0.01

- Means bearing different superscripts (a, b, c, d) within row differ significantly (P<0.05)
- Where: T1= Banana based milk smoothie
  T2= 75% Banana and 25% red plum based milk smoothie
  T3=50% Banana and 50% red plum based milk smoothie
  T4=25% Banana and 75% red plum based milk smoothie

Table 4 Sensory profile of banana and red plum based milk smoothies (Means±SE)

Sensory attributes	Banana and red plum based milk smoothies			
	T1	T2	T3	T4
Colour & Appearance	7.08^{a}±0.13	5.85^{b}±0.35	7.20^{a}±0.17	5.58^{b}±0.34
Flavour	7.20^{ab}±0.17	6.04^{b}±0.35	7.29^{a}±0.17	5.66^{b}±0.37
Body & Texture	7.08^{ab}±0.17	4.55^{c}±0.86	7.20^{a}±0.20	6.78^{abc}±0.20
Sweetness	6.33^{a}±0.38	5.12^{b}±0.28	6.58^{a}±0.32	5.16^{b}±0.27
Acidity	6.87^{a}±0.20	5.08^{c}±0.26	6.95^{a}±0.22	6.04^{b}±0.35
Consistency	6.33^{a}±0.20	5.41^{b}±0.26	6.41^{a}±0.19	5.14^{b}±0.21
Mouthfeel	6.70^{a}±0.23	6.16^{b}±0.23	6.70^{a}±0.23	6.45^{b}±0.35
Overall acceptability	6.79^{ab}±0.19	5.29^{d}±0.26	6.87^{a}±0.23	6.20^{bc}±0.18

- Means bearing different superscripts (a, b, c, d) within row differ significantly (P<0.05)
- Where: T1= Banana based milk smoothie
  T2= 75% Banana and 25% red plum based milk smoothie
  T3=50% Banana and 50% red plum based milk smoothie
  T4=25% Banana and 75% red plum based milk smoothie

mean values of pH in milk smoothies varied significantly between control and treatments Fig. 2a. The highest mean value of pH was in control while the lowest was in T4 groups. Milk smoothie prepared with the addition of red plum exhibited a significantly lower pH value than control which might be due to the lower pH of the red plum fruit (2.72-3.84) reported by Costa (2013) as compared to the banana fruit (4.68) by de Jesus et al. (2004). The titratable acidity value did not differ significantly (P>0.05) among all milk smoothie samples Fig. 2b. The highest mean value of titratable acidity was observed in T1 and lowest in T4. The variation in titratable acidity value in milk smoothie is due to the variation in the titratable acidity value of fruits. The mean values of specific gravity were in T1 (1.061±0.01), T2 (1.066±0.02), T3 (1.069±0.01), and T4 (1.071±0.01) respectively (Table 2) and did not differ significant (P>0.05). This might be due to the comparable specific gravity of added fruit in the milk smoothie.

Vitamin-C

The mean contents of Vitamin-C (mg/100 g) in T1, T2, T3, and T4 were 3.55±0.03, 4.98±0.05, 6.29±0.05, and 7.61±0.01respectively Table 2. The highest mean value of Vitamin-C was in T4 while the lowest was in T1. The analysis of variance indicated a significant (P<0.05) increase in mean Vitamin-C content with increased levels of red plum in the formulation. The increasing trend of vitamin-C with an increase in red plum in the smoothies could be attributed to the higher (25 percent) vitamin-C in red plum as compared to lower (10 percent) in banana. These findings are very well agreed with the reports of Shukla et al. (2017) on quality characterization of pasteurized mango based milk beverages.

Sensory profile of red plum milk smoothies

The mean scores of colour and appearance in T1, T2, T3 and T4 were 7.08±0.13, 5.85±0.35, 7.20±0.17 and 5.58±0.34 respectively Table 4. The highest mean score of colour and appearance was observed in T3 while lowest was in T4. The analysis of variance indicated no significant (P>0.05) difference between control (T1) and T3 but these scores were significantly (P<0.05) higher than T2 and T4. The mean scores of flavour in T1, T2, T3 and T4 were 7.20±0.17, 6.04±0.35, 7.29±0.17 and 5.66±0.37 respectively Table 4. The highest mean score of flavour was observed in T3 while lowest was in T4. The analysis of variance indicated significantly (P>0.05) higher mean flavour score of T3 than T2 and T4. The mean scores of body and texture were for T1 (7.08±0.17), T2

(4.55±0.86), T3 (7.20±0.20) and T4 (6.78±0.20) respectively Table 4. There was no significant (P>0.05) difference between control and T3 but these scores were significantly (P<0.05) higher than T2. The mean scores of sweetness in T1, T2, T3 and T4 were 6.33±0.38, 5.12±0.28, 6.58±0.32 and 5.16±0.27 respectively Table 4. The highest mean score of sweetness was observed in T3 while lowest was in T1. The highest mean score of acidity was observed in T3 while lowest was in T2. The analysis of variance indicated no significant (P>0.05) difference in mean acidity score between T1 and T3 but these scores were significantly (P<0.05) higher than T1 and T4. The mean scores of consistency in T1 (6.33±0.20), T2 (5.41±0.26), T3 (6.41±0.19) and T4 (5.14±0.21) were respectively Table 4. The mean scores of mouth feel in T1, T2, T3 and T4 were 6.70±0.23, 6.16±0.23, 6.70±0.23 and 6.45±0.35 respectively. Smoothie prepared with 50% banana and 50% red plum (T3) rated highest score for mouth feel and rated lowest score for T2. The mean scores for overall acceptability smoothie prepared with banana and red plum was rated for T1 (6.79±0.19), T2 (5.29±0.26), T3 (6.87±0.23) and T4 (6.20±0.18) respectively Table 4. Smoothie prepared with addition of 50% banana and 50% red plum showed significantly (P<0.05) higher OA scores than T1 and T4; however C had comparable scores with T3 and T4. The sensory scores on most of the sensory attributes of best variant in all breeds overall showed comparable scores to the control. It showed that panelists were highly satisfied with the product of 50:50 percent banana and red plum. However, panelists recorded the significantly (P<0.05) higher scores on acidity in products than control. The similar pattern of acceptance was observed by (Bhardwaj, 2011) during utilization of fruits and vegetable in preparation of milk based beverage. Balaswamy et al. (2013) also advocated that the selection of fruit is not a single criterion for acceptance of smoothies but its acceptance on sensory basis is also dependant on the proportion of its use in smoothie recipe.

Conclusions

Red plum is highly rich in fibres, vitamin-C and phyto-active compounds. Results can be concluded that milk smoothie prepared with incorporation of red plum significantly increased the fiber and fat content in the milk products. Milk and milk products are deficient in vitamin-C however in this study on addition of red plum in milk smoothie considerably increased the vitamin-C. Milk smoothie prepared with the addition of 50: 50 proportions of banana and red plum rated best on the basis of sensory evaluation by sensory panelist. This study further recommended for the storage study of selected products with control.

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Indian J Dairy Sci 74(6): 498-503
https://doi.org/10.33785/IJDS.2021.v74i06.005

RESEARCH ARTICLE

Antimethanogenic effects of soybean straw and seaweed (*Sargassum johnstonii*) based total mixed ration in crossbred cows

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Received: 20 August 2021 / Accepted: 08 September 2021 / Published online: 21 December 2021

Abstract: Ruminants are responsible for 50 % of anthropogenic methane production. Methane has higher global warming potential which is 28 times more than CO_2. The present investigation was conducted to evaluate effect of supplementation of soybean straw and seaweed (*Sargassum johnstoni*) in the ration of dairy cows on methane mitigation and production performance of animals. Six crossbred lactating cows received three treatments in Switch Over Design for 135 days in 3 periods of 45 days each. The treatments consist of T1: TMR with compound concentrate mixture and wheat straw (60:40); T2: TMR with 20 % each of wheat straw and soyabean straw and 60 % compound concentrate mixture and T3: TMR (60:40) with seaweed (*Sargassum johnstonii*). Methane emission was estimated by SF_6 tracer technique. The DM and nutrient intake were at par in T1 and T2, but was significantly low in T3. The milk yield observed was also less in T3 than T1 and T2 which were at par. The methane production was significantly reduced by 20.79 % and 16.53% ($P<0.05$) in soybean (T2) and seaweed (T3) supplemented group as compared to control group. The loss of dietary energy through methane also significantly decreased in T2 and T3 than T1. The results indicated that supplementation of soybean straw and seaweed in TMR has tremendous potential for methane mitigation in crossbred cows. However, further research is needed to ameliorate the feed and nutrient intake by increasing palatability of seaweeds based TMR.

Keywords: Feed efficiency, Methane, Milk yield, Seaweeds, Soybean straw, TMR

Introduction

Methane (CH_4) is a principal source of greenhouse gas emission from enteric fermentation in ruminants (Opio, et al. 2013). In India, per capita methane emission is higher as compared to developed countries (Johnson et al. 2002) due to poor quality roughages and less productivity of animals. Indian livestock emits 9.253 Tg enteric methane annually. Cattle is the largest producer with 4.92 Tg, contributing to 56% of total emission in the country (ICAR-NIANP, 2018). In addition, ruminants loose between 2 to 12 % of the dietary gross energy in the form of CH_4 (Johnson and Johnson, 1995). Different strategies are used to mitigate methane emission of which dietary manipulation is easy and economical. It involves a selection of feeds with secondary metabolites like tannins and saponin (tree leaves, legume straws, babul pods, lucerne etc.), feeds rich in halogenated compounds like seaweeds and processing of feeds and fodder into total mixed ration etc. for abatement in rumen methane emission (Beauchemin et al. 2020).

Seaweeds are macroalgae which are classified as brown algae (*Phaeophyceae*), red algae (*Rhodophyceae*) and green algae (*Chlorophyceae*) on colour basis (Makkar et al. 2016). More than 7500 km of Indian coastline is potential environmental province for growth of seaweeds in Tamil Nadu, Gujarat coast, Lakshadweep and Andaman Nicobar Islands. Recent studies have revealed high antimethanogenic potency of seaweeds in *in vitro* (Maia et al. 2016; Molina-Alcaide et al. 2017; De la Moneda et al. 2019) and *in vivo* with different species (Li et al. 2018; Roque et al. 2019; Kinley et al. 2020). Seaweed supplementation also has positive results on performance of lactating animals with increasing milk yield (Bendary et al. 2013; Singh et al. 2015). Legume forages also help in methane mitigation in ruminants, which is often explained by the presence of condensed tannins, low fibre content, high dry matter intake, and quicker rate of

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passage from the rumen (Beauchemin et al. 2008). Legume straw supplementation significantly reduces production of methane in *in vitro* and *in vivo* compared to cereal straws (Prajapati, 2016; Sherasia et al. 2018; Jasvantgiri, 2019). Milk yield also increases when 50 % of cereal straw is replaced by legume straw in dairy animals (Islam et al. 2020). Present study aimed to investigate effect of soybean straw and seaweed (*Sargassum johnstonii*) based Total Mixed Ration (TMR) on methane mitigation and performance of crossbred dairy cows.

Materials and Methods

Experimental design and animals

The experiment was conducted on 6 HF × Kankrej (50:50) crossbred dairy cows randomly allotted to three treatments in a 3 × 3 Switch Over Design. There were three periods each of 45 days duration. During the experiment, one animal suffered from mastitis and hence removed from experiment. So, results were compiled from the data of 5 animals in each period. Permission was granted by Institutional Animal Ethics Committee (IAEC/310/ANRS/2019) to conduct the experiment. *Sargassum johnstonii* dried biomass was procured from local vender in Veraval coast in Gujarat. It was included @ 8% inclusion level based upon *in vitro* studies (Katwal et al. 2020). The animals received three treatments viz; T1: TMR with compound concentrate mixture and wheat straw (60:40); T2: TMR with 20% each of wheat straw and soyabean straw and 60% compound concentrate mixture and T3: TMR (60:40) as T1 but DORB is replaced with 8 % seaweeds (*Sargassum johnstonii*). Molasses is added at different level to make TMR isocaloric and iso nitrogenous. TMR was prepared in TMR machine with grinding and mixing the different ingredients represented in Table 1.

DM and nutrient intake

The nutrient requirement of animals was met as per ICAR (2013) feeding standards. Daily feed intake was recorded by weighing the feed offered and the leftover of each animal. The DM and nutrient intake were calculated from feed intake. The nutrient composition of TMR is given in Table 2.

Milk yield and composition

Daily milk yield of cows was recorded during morning and evening at time of milking. 4% Fat corrected milk (FCM) and fat protein corrected milk (FPCM) yield were calculated as per NRC (2001):

$$FCM\ (kg) = 0.4\ (Milk\ Yield) + 15\ (Fat\ Yield)$$

$$FPCM\ (kg) = Milk\ production\ (kg) \times (0.337 + 0.116 \times fat\ \%) + 0.06 \times protein\ \%$$

Table 1 Ingredient composition (%) of total mixed rations

Ingredient	T1	T2	T3
Wheat straw	40.00	20.00	40.00
Soyabean straw	0.00	20.00	0.00
Compound Con. Mixture	46.0	40.0	46.0
DORB	8.00	10.0	0.00
Seaweed	0.00	0.00	8.00
Molasses	5.00	9.00	5.00
Mineral mixture with salt	1.00	1.00	1.00
Feed cost (₹)	1467.44	1468.6	1613.04

Table 2 Chemical composition of total mixed rations (% on DM Basis)

Parameters	T1	T2	T3
Dry matter	91.98	92.00	91.90
Organic matter	85.74	85.72	81.98
Crude Protein	12.46	12.51	12.80
Ether Extract	3.78	3.91	3.70
Crude Fibre	25.70	25.54	24.82
Total Ash	14.26	14.29	17.94
Acid Insoluble Ash	8.69	5.58	8.34
Nitrogen Free Extract	43.8	43.7	40.7
NDF	66.28	64.55	63.24
ADF	43.04	41.41	42.45
Hemicellulose	23.24	23.12	20.78
Cellulose	23.07	23.54	27.48
Lignin	7.13	7.90	7.39

About 100 ml of milk samples were collected in clean plastic bottles in the morning and evening at fortnightly intervals and were mixed. Analysis of milk composition was done using Lactoscan milk analyser. Milk samples were analysed for Total Solid (%), Fat (%), Solid Not Fat (%), Protein (%), Lactose (%) and Salt (%).

Methane emission and energy balance

Methane emission was measured by collecting breath samples for three consecutive days using sulphur hexafluoride (SF_6) tracer technique. Small permeation tubes (PT) were filled with pure (99.9%) SF_6 gas under liquid nitrogen. After standardizing the SF_6 release rate, PT was inserted into the rumen of experimental animals through mouth. The breath samples of all crossbred cows were analysed for CH_4 and SF_6 gases, using Thermo Fisher ceres 800 Gas Chromatography fitted with Porapack N column for CH_4 and molecular sieve 5A for SF_6 analysis (Johnson et al. 1994). The column temperature was maintained at 50 ºC and nitrogen was used as a carrier gas, with flow rate of 30 ml/min. Energy content of CH_4 was considered as 13.34 Kcal/g. All the samples were analysed in triplicate and the CH_4 emission rate was calculated as:

$$Q\,CH_4 = Q\,SF_6 \times (CH_4)/(SF_6)$$

Where, $Q\,CH_4$ = Methane emission rate (g/min), $Q\,SF_6$ = Known release rate of SF_6 from permeation tube (g/min), CH_4 = Methane concentration of collected sample in canister (µg/m^3) and SF_6 = SF_6 concentration of collected sample in canister (µg/m^3).

Energy intake was calculated from TDN intake obtained by conducting digestibility trial on each animal in each period.

Feed efficiency and economics

Feed efficiency was determined as the amounts of DM, DCP and TDN intake per kg milk yield and amount of DMI per kg 4% FCM and FPCM. The feeding cost was calculated from records of daily feed consumption and procurement price of feeds and fodder used in the experiment, based on that economics of milk production was calculated.

Statistical analysis

The experimental data were analysed by analysis of variance using General Linear Model procedure as per the methods of Snedecor and Cochran (1994), with the help of SAS software programme.

Results and Discussion

DM and nutrient intake

The average daily DMI of crossbred cows in T1, T2 and T3 groups was 12.56, 12.25 and 11.26 kg, respectively which was significantly (P=0.0004) low in T3 (seaweed group). The DMI declined by 10.35% in cows fed seaweed as compared to control. The higher reduction in DMI of T3 cows may be due to less palatability of seaweed containing TMR which was less consumed by animals during last 2 weeks of each period. Less DMI resulted in significant low intake of CP, DCP and TDN in T3 than T1 and T2. Roque et al. (2019) observed significant ($P < 0.001$) decrease in dry matter intake of Holstein cows by 10.8 and 38.0% at low (0.5%) and high (1%) level of *Asparagopsis armata* inclusion, respectively compared to control group. However, Singh et al. (2015) incorporated brown seaweed (*Sargassum wightii*) in the diet of lactating Sahiwal cows to the extent of 20% in concentrate mixture without significant difference in DMI among treatment groups. Similar to present findings, no significant difference on DMI of crossbred cows was observed when soybean straw replaced wheat straw up to 50 and 75% level in diet of crossbred cows (Mudgal et al. 2010). No adverse effect of replacing 50% of wheat straw by groundnut straw in TMR was observed in cattle (Sherasia et al. 2018; Jasvantgiri, 2019) and buffalo (Prajapati, 2016).

Milk yield and composition

The data of milk yield and composition are given in Table 3. Daily milk yield was 23.79% lower in T3 than T1 group, which was due to less DM and nutrient intake in T3 group. The average FCM and FPCM yield (kg/d) also reduced by 21.22% and 20.65% in T3 as compared to other groups due to lower milk yield. There was

Table 3 Milk production and composition

Parameter	T1	T2	T3	SEM	P value
Milk yield (kg/d)	8.88	9.07	7.17	0.50	0.0725
FCM yield (kg/d)	8.51	8.50	7.02	0.45	0.1020
FPCM yield (kg/d)	7.01	7.01	5.81	0.36	0.0982
Fat (%)	4.01	3.75	4.03	0.13	0.7804
SNF (%)	8.59	8.54	8.50	0.05	0.5958
Protein (%)	3.07	3.05	3.02	0.01	0.1819
Lactose (%)	4.70	4.67	4.62	0.01	0.0768
Salts (%)	0.70	0.69	0.69	0.004	0.6594
Total solids (%)	12.60	12.30	12.53	0.16	0.8113

no significant difference among all parameters of milk composition in all three treatments. The present findings are in agreement with Mudgal et al. (2010) who reported no significant effect of soybean straw on milk yield in crossbred cows when wheat straw was replaced at 50 and 75% level. Khare et al. (2018) reported that soybean straw could be supplemented upto 20% level without any adverse effect on milk production. Similarly, for seaweeds also Roque et al. (2019) reported 11.6 % reduction in milk yield in cows fed concentrate mixture with 1% *A. armata* compared to control (P<0.001). However, Hong et al. (2015) reported no significant difference in milk yield of Holstein cows with different levels (0%, 2%, and 4%) of Brown Seaweed By-Products (BSB). Singh et al. (2015) reported significant increase in milk yield with incorporation of 20% *Sargassum wightii* in concentrate mixture of lactating Sahiwal cows. The decrease in milk yield in present study was due to low DM and nutrient intake in seaweed group which can be taken care by improving DM and nutrient intake by addressing palatability of seaweed.

Methane emissions

The data pertaining to methane emission is depicted in Table 4. The daily CH_4 emission (g/d) was 236.89, 187.64 and 197.73 g/day. The daily CH_4 emission (g) significantly reduced by 20.79% and 16.53% (P<0.05) in soybean and seaweed supplemented group respectively, as compared to control. Methane emission (g/kg milk) significantly reduced by 22.42% (P<0.05) in soybean supplemented group as compared to control, however, in T3 due to less milk yield, it was at par with T1 in spite of low daily methane emission. The results indicated that the feeding soybean straw based TMR as well as TMR with supplementation of seaweed has remarkable potential for methane mitigation in crossbred cows. Sherasia et al. (2018) reported significant reduction in enteric methane emission by 7.79% (g/day) and 15.13% (g/kg DDMI) in Kankrej cows fed groundnut straw based TMR as compared to only wheat straw based TMR. Chaudhari (2018) also observed 10.5% reduction (P<0.001) in methane emission in crossbred calves offered TMR (50:50) with pigeon pea straw replacing 50% of wheat straw in TMR. Similarly, Roque et al. (2019) observed that methane production (g/d) and g/kg DMI decreased significantly (P<0.0001) by 26.4 and 20.3% at the low (0.5%) level of *A. armata* inclusion and by 67.2 and 42.7% at the high (1%) level of inclusion. Methane intensity (g/kg milk yield) significantly decreased by 26.8 and 60% from cows fed at 0.5% and 1.0% of *A. armata* inclusion level. Our results also revealed reduction in methane due to seaweed; however, due to decrease in milk yield, methane (g/kg milk) was higher.

Energy intake and loss of energy as methane

The energy intake in form of GE, DE, ME and NE was calculated from TDN intake and was found lower in T3 due less DMI. Energy loss (Mcal/d) in form of CH_4 was lower in T2 (20.63%) and T3 (16.50%) compared to T1. The energy loss in form of CH_4 as % of NE intake was 16.73% less in T2 than T1 (Table 4). Hence, in spite of less NE intake in T2, milk yield was at par with T1 as the dietary

Table 4 Methane emission and energy loss in crossbred cows

Parameter	T1	T2	T3	SEM	P value
Methane emission					
CH_4 (g/d)	236.89[a]	187.64[b]	197.73[b]	10.70	0.04
CH_4 (g/DMI)	18.64	15.51	18.93	0.95	0.08
CH_4 (g/DDMI)	36.34	31.80	35.62	1.89	0.28
CH_4 (g/kg milk)	26.71[a]	22.52[b]	29.03[c]	2.35	0.04
Energy Intake (Mcal/d)					
GE	32.59[a]	31.97[a]	27.08[b]	0.83	0.01
NE	13.63[a]	12.98[a]	11.05[b]	0.34	0.01
Energy loss					
Through CH_4 (Mcal/d)	3.15[a]	2.50[b]	2.63[b]	0.14	0.04
Through CH_4 as % of GE intake	9.67	8.14	9.87	0.50	0.22
Through CH_4 as % of NE intake	23.12	19.25	23.51	1.21	0.25

Table 5 Feed efficiency and economics

Parameter	T1	T2	T3	SEM	P value
Milk yield (kg/kg DMI)	0.71	0.74	0.63	0.03	0.2275
FCM yield (kg/kg DMI)	0.68	0.69	0.62	0.03	0.3452
FPCM (kg/kg DMI)	0.56	0.57	0.51	0.02	0.3524
Economics					
Feed cost (₹ /d)	203.90	195.71	195.36	2.83	0.138
Feed cost (₹ /kg milk)	22.93	22.50	28.85	1.67	0.069
Income from sale of Milk (₹ /d)	252.13	264.73	210.43	16.04	0.072
Return over feed cost (₹ /d)	51.91	64.72	15.68	14.21	0.060

energy saved through methane mitigation supported the milk production. In seaweed group (T3), though dietary energy loss through methane was saved by methane mitigation, it could not support the milk yield because, NE intake was significantly less due to less DM and nutrient intake. Sherasia et al. (2018) in Kankrej cows, Prajapati (2016) in Surti buffalo and Chaudhari (2018) in crossbred calves reported significant reduction in energy loss through methane when 50% of wheat straw in TMR was replaced by legume straws.

Feed efficiency and economics

The feed efficiency and economics are given in Table 5. There was no significant difference among treatment groups, however feed efficiency was higher in T1 and T2 group as compared to T3. Feed cost per kg milk production was lower in T2 and T1 as compared to T3 due to less daily milk yield in T3. The sale price of milk was calculated on the basis of minimum and maximum fat and SNF observed during the experiment with Rs. 29.38, 28.32 and 29.26 in T1, T2 and T3, respectively. The return over feed cost was higher in T2 (24.67%) and lower in T3 (69.79%) compared to T1 due to difference in milk production among the groups. Similar to our findings, less feed cost and higher net profit was observed in crossbred dairy cows offered pigeon pea straw (Chetan et al. 2017) or groundnut straw (Islam et al. 2020) replacing cereal straw. Bendary et al. (2013) reported non-significant difference in feed efficiency and economics of feeding in cows fed seaweed @ 50g/head/day as compared to control group. Sharma and Datt (2020) also observed non-significant difference in feed efficiency for milk yield in dairy cows supplemented with red seaweed based powder (*K. alvarezii*: *G. salicornia*: *K. alvarezii* in 1: 1: 1 ratio) @ 1.5 and 3% of ration.

Conclusions

Incorporation of soybean straw @ 20% level in TMR had no adverse effect on animals' performance, helped in reducing methane emission and saving loss of dietary energy through methane. Inclusion of *S. johnstonii* @ 8% in the ration reduced DM and nutrient intake, milk yield but helped in methane mitigation. Hence, further research is required to decide the optimum inclusion level of *S. johnstonii* and measures to ameliorate its palatability to maintain DM and nutrient intake so as to exploit the antimethanogenic potential.

Acknowledgement

Authors are highly thankful to the ICAR for funding the Outreach project on Methane as well as NIANP, Bangalore and Anand Agricultural University for providing necessary guidelines and facilities to carry out the present study.

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Indian J Dairy Sci 74(6): 504-508
https://doi.org/10.33785/IJDS.2021.v74i06.006

RESEARCHARTICLE

Lactoferrin gene polymorphism of exons 8 and 13 in Murrah buffalo

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Received: 11 November 2021 / Accepted: 12 December 2021 / Published online: 22 December 2021

Abstract: Lactoferrin is one of the important candidate genes for mastitis resistance in dairy animals. The gene is located on chromosome BBU21 and consists of 17 exons spanning over 32.95 kb of genomic DNA. The present study was undertaken to identify allelic variants in exon 8 and 13 of lactoferrin gene and their association with incidence of clinical mastitis in Murrah buffalo. A total of 200 lactating Murrah buffaloes, grouped as mastitis affected and non-affected, were included in the study. Genomic DNA was isolated from the whole blood sample of each animal. Two primer sets were used to amplify exon 8 and 13 of lactoferrin gene, which yielded respective amplicons of 216 bp and 211 bp. The polymerase chain reaction-restriction fragment length polymorphism analysis of lactoferrin gene revealed monomorphic pattern in exon 8 and polymorphic pattern in exon 13. *Hpy 188I*-RFLP for exon 13 exhibited polymorphism with three genotypes: AA, AB and BB with respective frequencies of 0.20, 0.58 and 0.22 whereas, frequencies for A and B alleles were estimated as 0.49 and 0.51. Comparison of nucleotide sequence of exonic region of lactoferrin gene in Murrah buffalo with that of *Bos taurus* cattle revealed a total of 5 mutations out of which 2 were transition and 3 were transversion. The SNPs in exon 8 were found to be non synonymous and revealed two amino acid changes in exon 8 of Murrah buffalo as compared to *Bos taurus* cattle. Chi-square ($\chi 2$) analysis indicated non significant association between genetic variants of exon 13 and incidence of clinical mastitis.

Keywords: Lactoferrin, Murrah buffalo, PCR-RFLP, Single nucleotide polymorphism

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Introduction

Among various health disorders in dairy animals, mastitis is one of the most expensive and devastating diseases in dairy animals including buffaloes. Mastitis is the inflammation of the mammary glands caused predominantly by entry through the teat by certain bacteria especially Streptococcus, Staphylococcus and Escherichia etc. It causes reduced milk yield, poor milk quality and lactation persistency and early culling contributing to huge economic losses to dairy farmers. In India, about 1 to 10% and 5 to 20% of buffaloes are affected with clinical and subclinical mastitis respectively every year (Joshi and Gokhale, 2006). Selective breeding of buffaloes for increased resistance to mastitis is difficult as it is polygenic trait with very low heritability. Earlier mastitis was considered purely a managemental disease, but at present several candidate genes (lactoferrin, BoLA-DRB-3, CARD15, interleukins, FEZL, CD14, etc.) have been identified for mastitis resistance. Lactoferrin is an important candidate gene having relation with the innate immunity and is considered to be a promising candidate gene in selection for mastitis resistance (Seyfert et al. 1996). It is a minor whey non-heme iron binding protein with molecular weight of 80 kDa containing a single polypeptide chain of 708 amino acids. The gene is located on chromosome BBU21 and consists of 17 exons spanning over 32.95 kb of genomic DNA. It is a potent activator and regulator of various immunological functions such as granulopoiesis, in vitro antibody synthesis, natural killer cell cytotoxicity, lymphocyte proliferation, complement activation and production of interleukins (Sanchez et al. 1992); (Kimber et al. 2002). Analysis of genetic polymorphism in lactoferrin gene and its relationship with udder infections has practical significance in marker-assisted selection (MAS) in dairy animals to maximally exploit their genetic potential for milk yield. Identification of lactoferrin variants as a genetic marker associated with mastitis resistance in buffalo would allow producers to decrease costs associated with mastitis by improving herd health through genetic selection. The polymorphism in lactoferrin gene and its association with mastitis has been described in *Bos taurus* (Li et al. 2004), but little information is available for exons 8 and 13 of lactoferrin gene in Murrah buffalo except for its promoter and 5' flanking regions (Kathiravan et al. 2010). Murrah breed of buffaloes is categorized among the best dairy breeds of riverine buffaloes, but its true

yield potential and economic contribution are hampered by increasing prevalence of mammary infections. Hence, the present study was undertaken with the objectives to identify polymorphism in exons 8 and 13 of lactoferrin gene through PCR-RFLP and its association with incidence of clinical mastitis in Murrah buffalo.

Materials and Methods

The experimental animals for the present study were taken from dairy herd of Murrah buffalo maintained at National Dairy Research Institute, Karnal, India. A total of 200 animals were sampled to identify polymorphism in exons 8 and 13 of lactoferrin gene. The animals were classified into affected and non-affected groups on the basis of past history of incidences of clinical mastitis from the treatment records of the herd. Among all, 50% of animals were mastitis-affected, while the remaining 50% were non-affected. Under sterile conditions, 10 ml of venous blood was collected from the jugular vein of buffalo in a 15 ml vacutainer tube containing 0.5 ml of 0.5M EDTA solutions, as an anticoagulant. Phenol-chloroform extraction method, as described by Sambrook and Russell (2001) with minor modifications was used for DNA isolation. The lactoferrin gene primers both forward (P1) and reverse (P2) for coding region of exons 8 and 13 were taken from published literature of (Li et al. 2004) and (Kathiravan et al. 2010) respectively (details of oligonucleotide sequence, annealing temperature and amplicon size are presented in Table 1). PCR amplification was carried out in programmed thermal cycler comprising final reaction volume of 25 µl containing 3 µl (100 ng) genomic DNA, 12.5 µl 2X PCR Master Mix (Fermentas), 0.5 µl of each primers and 8.5 µl nuclease free water. Amplification was performed using initial denaturation at 95°C for 2.5 minutes followed by 35 cycles of 94°C for 30s, respective annealing temperature for 30s and 72°C for 1 minute, with a final extension for 5 minutes at 72°C. PCR products were used for sequencing of lactoferrin gene through sequencing service provided by M/s. SciGenom Labs Pvt. Ltd. Basic Local Alignment Search Tool (BLAST) analysis was performed to find out sequence identity of lactoferrin gene of Murrah buffalo with other species. For determining the single nucleotide polymorphism (SNPs) in exons 8 and 13 of lactoferrin gene in Murrah buffalo, the available sequence in the NCBI for *Bos taurus* (Accession number-000179.1) was compared and aligned with the edited sequences of Murrah buffalo using ClustalW software.

PCR-RFLP condition

The preliminary selection of restriction enzymes to be used was done using NEB cutter V2.0 by submitting sequences obtained after amplification of experimental samples. PCR amplification of exons 8 and 13 revealed amplicon size of 216 and 211 bp by agarose gel electrophoresis. Amplified PCR products of exons 8 and 13 were digested using restriction enzymes *Hpa*II, *Hae*III and *Hpy*188I, *Hinf*I respectively. For restriction enzyme digestion, the reaction mixture comprised of a final volume of 20 µl containing 0.3 µl (10 U /µl) RE, 2.0 µl buffer, 7.70 µl milli Q water and 10 µl PCR product. Incubation was done for 10-12 hours at 37°C for all enzymes. Restriction enzyme digested products were separated by 2.5-3% agarose gel electrophoresis and visualized with ethidium bromide staining (@ 2µl/100 ml of gel) under UV light with a Gel Doc system (BioRad).

Statistical analysis

Chi-square statistic was used to analyze differences among genotypes and to assess significant association for susceptibility or resistance to mastitis. The chi square statistic ($\chi 2$) was calculated by the formula $\chi 2=\Sigma$ (Observed " Expected)^2 / Expected (Snedecor and Cochran, 1994). The observed genotype frequencies based on RFLP patterns were tested for Hardy-Weinberg Equilibrium (HWE) by chi-square test. The observed and expected frequencies were analyzed for goodness of fit at probability pd"0.05. The calculated chi-square value was compared with table p value to ascertain the significance of association between genotype frequency and mastitis incidence.

Results and Discussion

In the present study polymorphism was detected in exon 13 of lactoferrin gene through PCR-RFLP. Exons 8 and 13 of lactoferrin gene were amplified successfully which yielded amplicon size of 216 and 211 bp. The PCR-RFLP was performed on the amplified fragment of exons 8 and 13. Restriction enzymes (REs) *Hae*III and *Hpa*II were used for digestion of exon 8 having single cutting site. PCR-RFLP analysis of exon 8 revealed monomorphic pattern (AA) with fragment size of 122 and 94 bp using *Hae*III restriction enzyme. Similarly *Hpa*II restriction enzyme had single cutting site, producing two fragments of 125 and 91 bp (BB) that exhibited monomorphic pattern. (Fig.1 and 2).

The RE digestion for exon 13 was carried out by using *Hpy 188I* and *Hinf*I. Restriction enzyme *Hpy 188I* for 211bp amplicon revealed polymorphic pattern with three genotypes : AA (211 bp), AB (211, 164, and 47 bp), and BB (164 and 47 bp) with respective frequencies of 0.20, 0.58 and 0.22 (Fig. 3). Allelic frequencies of A and B genotype were estimated as 0.49 and 0.51, respectively. Genotypic frequency of AB heterozygote was more than the homozygote animals. However, restriction enzyme *Hinf*I revealed monomorphic pattern with band size of 130, 81 bp (CC) in exon 13. Kathiravan et al. (2009) performed PCR-SSCP analysis for bubaline lactoferrin gene and revealed monomorphic patterns in exons 2, 11 and 14. In another study Kathiravan et al. (2010) identified polymorphisms in exons 6, 7, 13 and their flanking intronic regions in the bubaline lactoferrin gene by PCR-SSCP analysis and revealed two SSCP variants with the frequencies of 0.92 and 0.08 in exon 13 of lactoferrin gene. Similarly Khatibi et al. (2013) reported four SSCP patterns with frequencies of 0.341, 0.259, 0.118 and 0.282 for A, B, E and F alleles respectively in

Table 1 Oligonucleotide Sequence, annealing temperature and amplicon size of Exons 8 and 13 of lactoferrin gene in Murrah Buffalo

Primer	Sequence (5'-3')	Annealing Temp.	Amplicon size (bp)
Exon 8	F- CTCTACCACTGACATCATAAT		
	R-CACTTTCCCTGAGGTTCTTC	54.0°C	216
Exon13	F-AGAGCTGGCTCCCCATGTTTCTT	58.5°C	211
	R-AGGGCCCTGTCCTGATGAAGC		

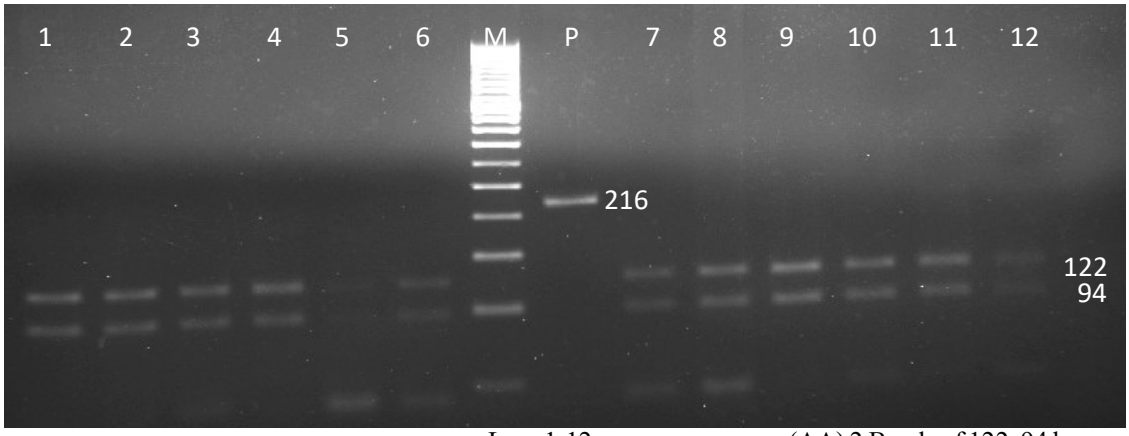

Fig 1. PCR-RFLP of Exon 8 of Lactoferrin Gene Using *Hae*III Restriction Enzyme

Lane 1-12 : (AA) 2 Bands of 122, 94 bp
Lane M : Marker (50 bp)
Lane P : PCR Product (216 bp)

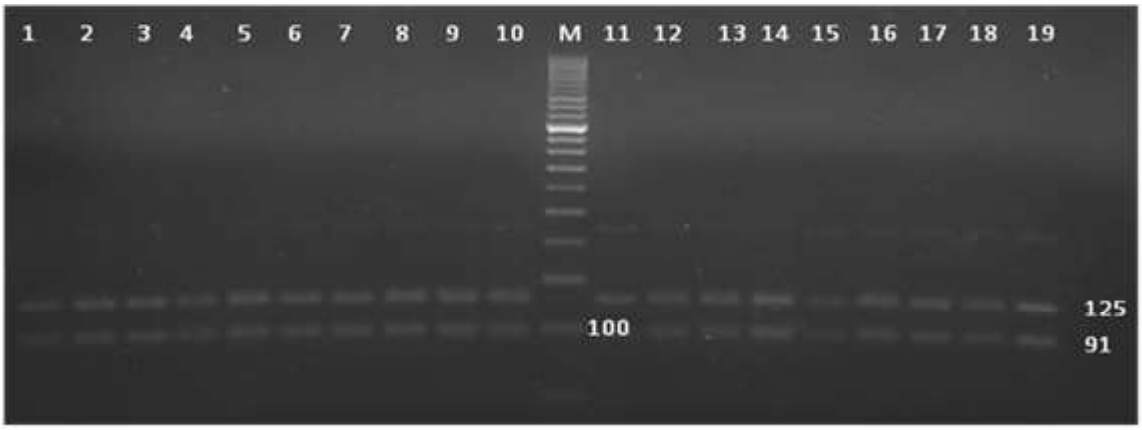

Fig 2. PCR-RFLP of Exon 8 of Lactoferrin Gene Using *Hpa*II Restriction Enzyme

Lane 1-19 : (BB) 2 Bands of 125, 91 bp
Lane M : Marker (50 bp)

lactoferrin gene in 85 Iranian buffaloes. Wojdak-Maksymiec et al. (2013) studied lactoferrin polymorphism in 588 Holstein cows and reported two genotypes AA and AB with respective frequencies of 0.568 and 0.432. However, Bukhari et al. (2015) observed monomorphic pattern in promoter region of lactoferrin gene using *Taq* 1 restriction enzyme in Jersey crossbred cattle. However, Dinesh et al. (2015) reported polymorphism in exon 7 and 12 of lactoferrin gene in Murrah buffalo. Singh et al. (2016) reported monomorphic pattern in exons 2, 3, 14 and their flanking intronic regions of lactoferrin gene in Deoni cattle by PCR-SSCP.

Association analysis was performed by chi-square test. For exon 13 with Hpy188I RE, calculated $\chi 2$ (2.10) value is less than tabulated $\chi 2$ (5.99) value at 2 degrees of freedom and 5 % level of significance. Hence, AA, AB and BB genotypes do not differ significantly with respect to mastitis incidence The observed genotypic frequencies were tested for Hardy-Weinberg

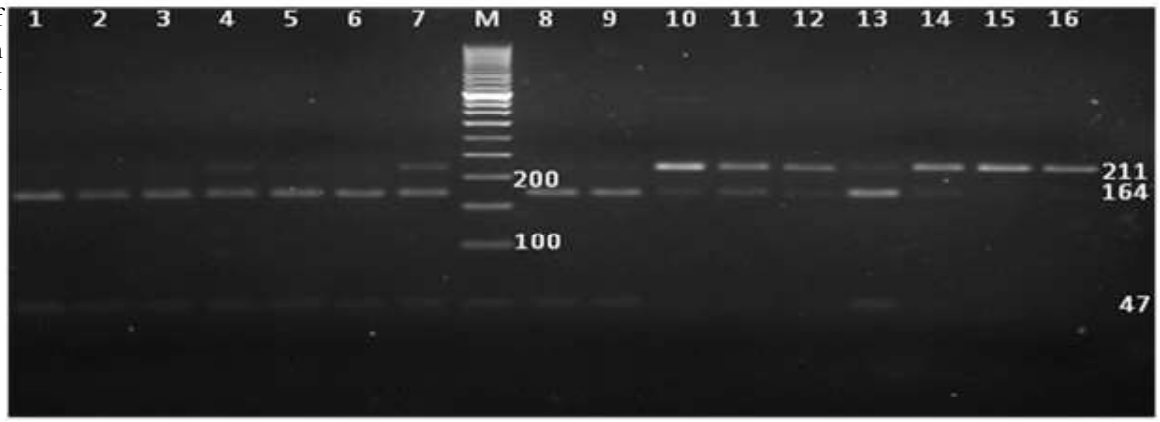

Fig 3. PCR-RFLP of Exon 13 of Lactoferrin Gene Using *Hpy*188I Restriction Enzyme

Lane 15, 16 : AA (211 bp)
Lane 4, 7, 10-14 : AB (211, 164, 47 bp)
Lane 1-3, 5, 6, 9 : BB (164, 47 bp)
Lane M : Marker (50 bp)

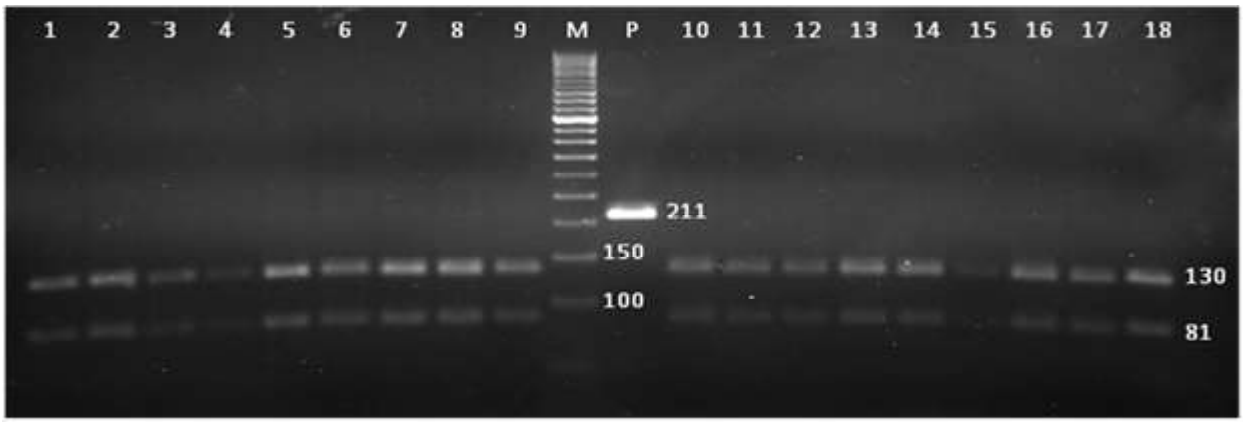

Fig 4. PCR-RFLP of Exon 13 of Lactoferrin Gene Using *Hinf*I Restriction Enzyme

Lane 1-18 : (CC) 2 Bands of 130, 81 bp
Lane M : Marker (50 bp)
Lane P : PCR Product (211 bp)

equilibrium and found that gene and genotypic frequency of exon 13 was in equilibrium, showing a non-significant association between genotype of exons 13. The results of present study are consistent with earlier reports of Kaminski et al. (2006) who reported non significant association between polymorphic variant in promoter region of lactoferrin gene and somatic cell count. Zhao et al. (2009) observed genetic polymorphism in promoter region of lactoferrin gene by PCR-RFLP using *Hinf*I and found that frequency of AA genotype was higher in healthy dairy cows, while BB genotype was found in dairy cows affected with sub-clinical mastitis. Huang et al. (2010) also reported non significant association between identified SNPs and somatic cell count. Similarly Dinesh et al. (2015) reported non significant association between genetic variant of exon 12 of lactoferrin gene and mastitis, however they observed significant association between genetic variant of exon 7 of lactoferrin gene and mastitis. Nanaei et al. (2016) did not find significant association between intron 6 of lactoferrin gene and 305 days milk yield, protein percentage, pregnancy length and milk days. In another study Dinesh et al. (2020) observed significant association between incidence of clinical mastitis and genetic variant of exon 6 and they reported that animals with AA genotype were found to be less susceptible to mastitis.

Comparison of nucleotide sequences of exonic regions of the lactoferrin gene with that of *Bos taurus* cattle by ClustalW multiple alignments revealed a total of five mutations. Two transitions and one transversion were observed in exon 8 of the Murrah buffalo lactoferrin gene as compared to cattle, while one transition and one transversion were found in exon 13 of lactoferrin gene in Murrah buffalo. The coding sequences were translated into amino acid sequence by using ExPAsy translate tools and the resulting amino acid sequence was aligned with corresponding sequence of *Bos taurus* by ClustalW. Conceptualized translation of nucleotide sequence of exon 8 revealed two amino acid changes in Murrah buffalo as compared to that of *Bos taurus* cattle. Amino acid changes observed in exon 8 were arginine to glycine and threonine to alanine at 303 and 346 position respectively. However, in exon 13 both the mutations were found to be synonymous in nature without

affecting the sequence of amino acid. Thus only the nucleotide changes in exon 8 were found to be non synonymous in nature affecting the sequence of amino acid In a similar study, Li et al. (2004) found polymorphisms in exons 4, 8, 9, 11, and 15 and in intron 4 in Holstein-Friesian cattle. A mutation occurring in exon 4 led to the amino acid substitution (isoleucine to valine), while other mutations were silent. O'Halloran et al. (2009) identified 47 polymorphisms in lactoferrin coding sequences. Out of these, 18 SNPs were synonymous causing no change in amino acid sequence, while 27 SNPs were associated with amino acid changes. The result of present study are in agreement with the earlier report of Kathiravan et al. (2010) who observed two point mutation in exon 13 of lactoferrin gene as compared to cattle.

BLAST results were used to check the percent homology of Murrah buffalo lactoferrin gene with that of other species. The sequence homology of exon 8 was 99% with *Bubalus bubalis*, 98% with *Bos taurus,* 98% with *Bos indicus*, 92% with *Capra hircus* and 92% with *Ovis aries*. Similarly, the percent homology of exon 13 was 100% with *Bubalus bubalis*, 98% with *Bos taurus,* 97% with *Capra hircus* and 96% with *Ovis aries.* BLAST results revealed that exonic region of lactoferrin gene in Murrah buffalo was 92 to 100% identical with several species. This is consistent with the finding on exonic region of the bubaline lactoferrin gene as reported by Kathiravan et al. (2010). A similar homology (65-100%) in a gene sequence among different mammalian species was reported by Teng, 2002.

Conclusions

PCR-RFLP analysis in exon 13 of lactoferrin gene in Murrah buffalo using *Hpy*188I restriction enzymes revealed polymorphism with three genotypes (AA, AB and BB), whereas exon 8 exhibited monomorphic pattern. Genotype AB occured with higher frequency as compared to AA and BB. Multiple sequence alignment of Murrah buffalo with that of cattle showed five SNPs. SNP found in exon 8 were found to be non synonymous. BLAST results revealed that exonic region of lactoferrin gene in Murrah buffalo was 92 to 100% identical with other species.

Acknowledgement

The authors are grateful to the Director, NDRI, Karnal and Head, Animal Genetics and Breeding Division, NDRI for providing necessary research facilities.

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https://doi.org/10.33785/IJDS.2021.v74i06.007

RESEARCH ARTICLE

Effect of parlour relocation on behaviour and post-adaptation milkability of lactating dairy cows

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Received: 02 October 2021 / Accepted: 01 November 2021 / Published online: 22 December 2021

Abstract: The aim of study was to investigate the effect of parlour relocation on behaviour of lactating cows and to understand the effect of behaviour on post-adaptation milkability in dairy cows. Seventy-two crossbred cows initially milked in tandem milking parlour were relocated to herringbone parlour and observed for 45 milking sessions. Recording of behaviour was done for the individual animal as they entered the holding area till exit after completion of milking. The results showed that there was marked behavioural changes associated with the relocation of animals. Sharp decline in milk yield was observed on the day of relocation which improved in subsequent milking sessions. The mean cortisol level pre and post milking was significantly ($P<0.01$) high in novel parlour to which the animals were unfamiliar. The behaviour of animals post-adaptation in holding area and milking area was found to be significantly ($P<0.01$) associated with the milkablity traits in terms of milk yield and milk flow. The temperament score of cows significantly ($P<0.01$) affected their milk yield and milk flow characteristics. No definite trend was seen due to effect of elimination behaviour on milkability. The study concluded that although lactating dairy cows have tendency to adapt in a new system, shifting them in the midst of lactation may cause production losses. The cow behaviour post-adaptation in milking parlours having batch milking may serve as potent tool to select and split large herds, based on milk yield and milk flow rates.

Keywords: Cow behaviour, Dairy cow, Milk yield, Milkability

Introduction

Good design and facilities are required in animal units to take advantage of natural behaviour which facilitate easy movement of animals and reduce the number of negative interactions with the stock handlers. In general, a quiet and consistent handling helps in promoting better productivity and good animal welfare by reducing the level of fear and stress in animals (Grandin 2018; Coleman and Hemsworth 2014). On a well organized farm, the dairy cows are habituated to milking routine operations. However, they may be often exposed to number of situations on day to day basis, which may be either disturbing their routine or creating a transient stress in them (Breuer et al. 2000; Neisen et al. 2009; Sutherland and Huddart 2012). As a consequence, dairy cows are likely to show behavioral and physiological responses to these multiple stressors (Van Reenen et al. 2002; Eicher et al. 2007).

Milking of cows in an unfamiliar parlour or changing the parlour type and design for better milking, the animals undergo initial stress and has been implicated as one of the major aversions for relocated cows (Grandin 1998). Studies show that relocated cows are subjected to strange surroundings, noise, odours, stock and people which contribute to stress and potential performance losses (Keeling et al. 2002; Grandin 2003; Macuhova et al. 2008; Sutherland et al. 2012). Such situations are characterized by frequent vocalization, defecation, and urination incidences considered as indicators of fear or stress in cattle (de Passillé et al. 1995; Grandin 1998); and increased movement (stepping and kicking) considered as a sign of agitation (Grandin 1993). Soch et al. (1997) recorded production losses with a decrease in milk yield from 19.0 kg to 10.2 kg on the first day of milking after moving from stanchion-stall to free-stall housing. Bruckmaier et

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al. (1993) mentioned inhibition of milk letdown in dairy cows milked in an unfamiliar or novel environment. On the other hand, there are studies on cow behavior post-adaptation in milking area in conjunction to holding area that may possibly explain the variation in individual milkablity of cows (Gergovska et al. 2012; Ishiwata et al. 2005) to make important managemental decisions on large farms. In this attempt, the present work was aimed to study the effect of parlour relocation on behaviour of crossbred dairy cows and to understand the milkability of cows in relation to behavior in milking and holding area.

Materials and Methods

Animals and management

The study was conducted at the Livestock Research Center, ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana, India. Sixty lactating crossbred Karan Fries (HF X Tharparker) and twelve Karan Swiss (Brown Swiss × Sahiwal) cows in lactation 1 to 4 were taken for 45 milking sessions. The animals were kept under loose housing system and were monitored through an automatic animal identification system consisting of a neck transponder, portal identification antenna, system controller, and ALPRO Windows kit (DeLaval, ALPRO windows 6.90). Milking was done thrice daily, in morning 05-00 to 07-00 h, in afternoon 12-30 to 13-30 h and in evening 17-30 to 18-30 h. The cows were initially milked in tandem milking parlour post-calving and then relocated to herringbone milking parlour (Average number of days in milk 110.59; range 62 to 175 days). The tandem milking parlour (2 x 6) provided optimal space conditions for each individual animal, where the cow could stand on milking platform in a box measuring 2.50 m in length and 0.75 m in width during milking, unaffected from other animals. The herringbone milking parlour (2 x 8) had GI pipe frame that provided standing space for milking of 8 cows simultaneously in a row having inner space of 0.75 m, outer space of 1.50 m and total length of 10.6 m for batch milking. In both cases, the animals were taken to a holding area adjacent to the milking parlour. The time each cow spent in the holding area before milking varied from 10 min to 30 min. Once the cows were in the milking parlour the routine was pre-milking udder washing, fore-stripping, cluster attachment, and post-milking teat spray.

Recording of behaviour

Animal behaviour was monitored daily from the time animal entered the holding area till its exit after completion of milking. Video-recording was done by the help of IR network camera (Model:DS-2CD2032-I).The camera was enabled with infrared technology for true day and night vision. The image capturing was at 15 frames per second (FPS) @ 2048X1536 resolution. In the holding area, behavioral features which were observed were their orientation, movement, licking fellow animals and looking up. In the milking area, FSK response, temperament score of cows and elimination behaviour was recorded. The FSK response was recorded based on flinch, step, and/or kick (FSK) score on 4 point scale (Sutherland and Tops 2014) :1 = no hind foot movement, cow may flinch, shiver or do nothing at all; 2 = hind leg lifted no higher than 20cm, step or shuffle of a hind leg; 3 = hind leg lifted higher than 20cm, step or forward kick of a hindleg; 4 = backward kick of hind leg. The temperament score was assessed using 5-point scale (as proposed by Tulloh 1961). Other behavior were recorded based on their frequency of occurrence using time-sampling method at 5 min interval during six intermittent morning milking sessions.

Estimation of plasma cortisol level

Blood collection was done in eight animals before relocation, in tandem parlour before and after milking during morning milking session. The same animals were again selected for blood collection after relocation in herringbone (novel) parlour at day 0, 5, 10 and 15 days, pre and post milking during morning milking session. Samples were drawn in sterile heparinized vacutainer tubes from jugular vein puncture, posing minimum disturbance to the animal during collection. The samples were centrifuged at 3000 rpm for 20 min at 4 ^{0}C to separate the plasma. The plasma was aspirated and stored at -20 ^{o}C temperature until analysis. Plasma samples were analyzed for cortisol concentrations using commercially available enzyme immunoassay kit (Cortisol EIA kit Item No. 500360, Cayman Chemical Company, Ann Arbor, MI).

Statitistical analysis

All data were tested for constant variance and departures from normal distribution using the univariate procedure in SAS (SAS Inst., Inc., Cary, NC) and data lacking normality were transformed prior to analysis. Quasi-experimental design technique was used to compare the groups. Parameters with two groups were tested for significant difference in means using student t-test. The parameters having more than two groups were tested for significance using ANOVA. The correlations between cow behaviour at the time of milking and milkability traits were studied using the Spearman's rank correlation coefficient.

Results and Discussion

Relocation of cows to novel parlour:

There was marked increase in flinching, stepping and kicking behaviour (FSK response) on the day of introduction at day 0 (Figure 1). FSK response reduced on next day of milking which further reduced and seen in 25% cows at 30th day of relocation. The novel parlour (Herrinbone type) was associated with greater degree of FSK than the tandem parlour. Similarly, relocation of cows to novel parlour also led to more elimination behavior during milking on the day of introduction. This behaviour was significantly reduced on 3rd day and onward in these cows. There was greater vocalization in cows on the day of introduction which

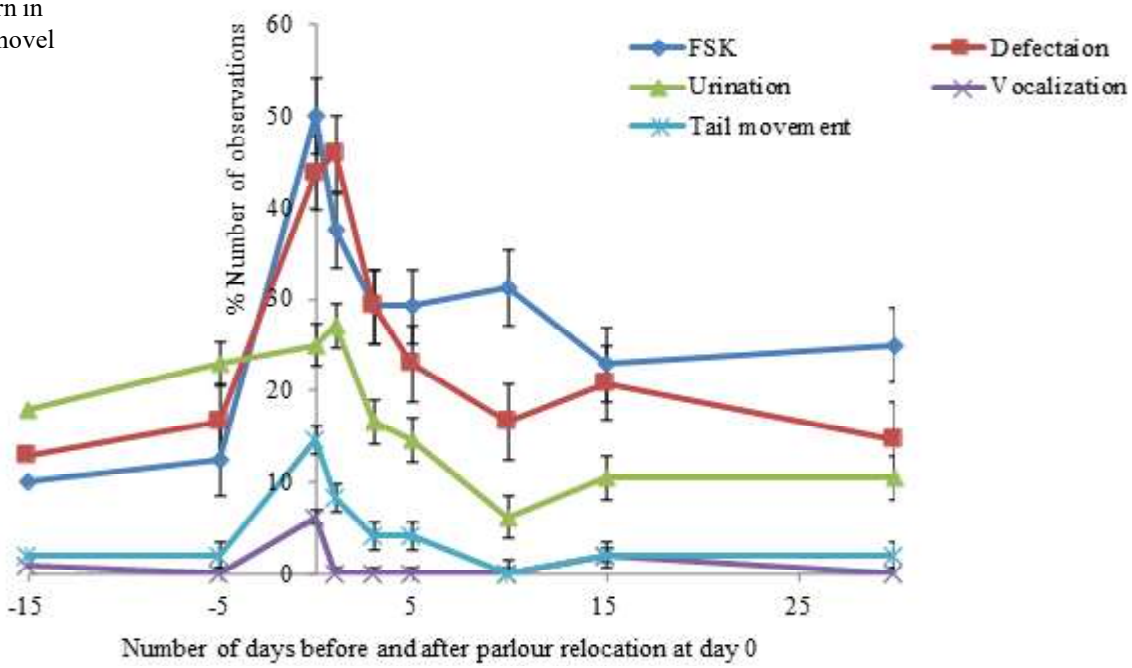

Fig. 1 Behavioural pattern in dairy cows relocated to novel parlour

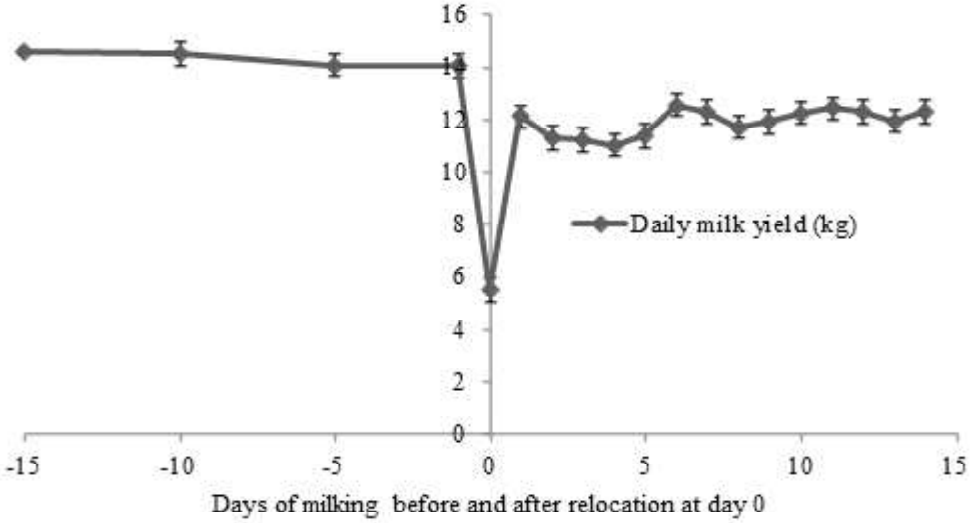

Fig. 2. Daily milk yield in cows relocated to novel palour

later reduced in subsequent milkings. Earlier studies on change in milking parlour environment or type of parlour suggest varying levels of discomfort and stress response in animals (Hopster et al. 2002; Wenzel et al. 2003; Gygax et al. 2008). Jacobs and Seigford (2012) reported changes in behaviour in dairy cows on day 0 of introduction in a novel parlour with a greater elimination and vocalization response and lowering of milk yield. However, the initial stress and discomfort with the milking process in the new system on d 0 subsequently subsided and the cows appeared to adapt within only a period of 24 h. Rushen et al. (1999) also reported increased incidences of defecation, urination, and vocalization in dairy cows in unfamiliar surroundings.

There was decrease in milk yield after being subjected to novel milking environment (Fig. 2) indicating acute stress in animals. The milk production in crossbred cows initially milked in separate milking boxes was 14.31±0.61 kg, which showed sharp decline on the day of introduction (5.51±0.5 kg). This may be due to social stress in herringbone parlour occurring from lesser individual space, with multiple cows milked simultaneously and adjacent to each other during milking. However, subsequent milking sessions showed improvement in milk yield but with variable yield. The milk yield was not stable up to 10th day of milking which later stabilized, however, the animals did not reach the same potential with which they produced earlier. Jacob and Seigford (2012) reported discrepancy in adaptation time may be due to differences

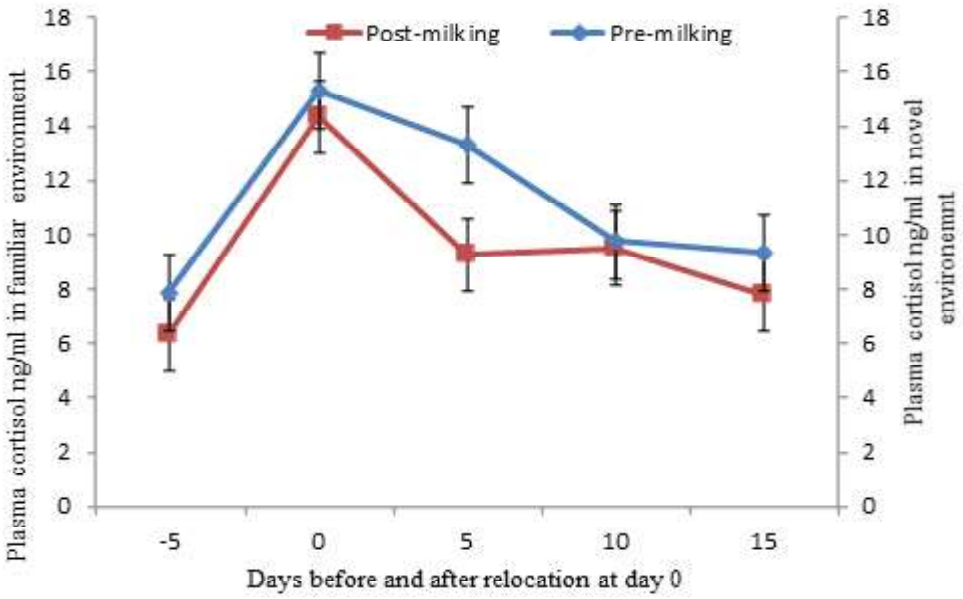

Fig. 3 Plasma cortisol concentration (ng/ml) in dairy cows before and after relocation

in milk production, milking frequency, milking environment and equipments used for milking. In the present study, the cows were seen to adapt quickly in the new system in less than 5 days, however, to achieve a stabilized production, at least 10 days time was taken. Further, since the animals were shifted in the midst of their lactation, it was found that such shifting of animals could affect their production. It may therefore be suggested from the study to get the animals acquainted for any changes made in milking environment before the lactation commences.

Measurement of cow stress (Cortisol estimation)

The mean level of cortisol in crossbred dairy cows are presented in Figure 3. The cortisol concentrations pre and post-milking in familiar milking environment differ significantly (P<0.01). When the cows were relocated to a novel milking environment (herringbone type), there was two fold increase in cortisol level pre-milking which was similar as post-milking. Later, as the cows were habituated, the level of cortisol reduced. The pre-milking and post-milking level of cortisol suggest reduced stress after milk removal. When the cows were brought in novel parlour, they suffered psychological stress which continued even after milking. Jacobs and Seigford (2012) reported similar findings in Holstein dairy cows subjected to automatic milking system from conventional parlours. Bruckmaier et al. (1993) reported cortisol level increase before milking was larger in novel environment than in the familiar environment, indicating the level of stress in cows. Sutherland et al. (2012) reported that in the familiar milking environment, serum cortisol concentrations were lower (P<0.05) in cows post-milking as compared to pre-milking values, but did not differ (P>0.05) pre- and post-milking in the novel environment regardless of cow temperament. Furthermore, they also classified the cows as high and low responders (based on their exit times from the crush) and found that cortisol concentrations prior to milking were higher (P<0.05) in high responders (exit time less than 2 s) than low responders (exit time of more than 4 s) cows in both the familiar and novel environments. Therefore, in our study it may be suggested that lactating dairy cows adapt quickly to being milked in a new system. Increased concentration of cortisol may be responsible for lower oxytocin levels in cows milked in unfamiliar environment, leading to poor milk letdown (Macuhova et al. 2002; Weiss et al. 2004; Sutherland et al. 2012). This may be the reason for decline in milk yield in unfamiliar milking environment in our study.

Effect of behaviour on milkability of cows post-adaptation

The milk yield (kg/ day) in cows which oriented towards the milking area was significantly (P<0.01) higher than those which oriented sideways and opposite to milking (Table 1). Likewise, the single session milk yield (morning) was also higher (P<0.01) for these animals. The cows having such tendency had significantly (P<0.01) high average and peak flow rates (1.10±0.01 and 2.21±0.02 kg/min) during milking. There was positive and significant correlation for milkability traits in crossbred cows based on their preference for orientation (Table 2). Similarly, the movement of cows in parlour for milking was also associated with higher milk yield (14.01±0.15 kg) and positive correlation (r = 0.528, P<0.01), with significantly higher average and peak flow rate (1.16±0.01 and 2.34±0.03 kg/min) (r = 0.428 and 0.422; P<0.01). Kumar et al. (2019) studied pattern of movement in Jersey crossbred cows and reported significant (P<0.01) variation in milk yield, milking time and milk flow rate based on exit score and parlour leaving speed (PLS). Gergovska et al. (2012) reported that for an efficient milking operation one should invest less time on the individual animal. In an another study, Suzuki et al. (1982) observed the movement of individual cows in the holding area

Table 1 Effect of cow behavior in holding and milking area on milkability (Mean±SE)

Parameter	Observations	No. of observations (%)	Total daily milk yield (kg)	Milk yield/session (kg)	Machine-on time (min)	Average milk flow (kg/min)	Peak milk flow (kg/min)
Holding area							
Orientation	Towards	48.1	13.18^{A}±0.15	6.13^{A}±0.13	5.55^{A}±0.06	1.10^{A}±0.01	2.21^{A}±0.02
	Sideways	22.1	11.95^{B}±0.17	5.30^{B}±0.15	5.06^{B}±0.07	1.05^{B}±0.01	2.11^{B}±0.03
	Opposite	29.8	11.89^{B}±0.16	5.12^{B}±0.14	5.03^{B}±0.06	1.03^{B}±0.01	2.08^{B}±0.03
Movement	Voluntary	50.4	14.01^{A}±0.15	6.83^{A}±0.13	5.88^{A}±0.06	1.16^{A}±0.01	2.34^{A}±0.03
	Forced	49.6	10.66^{B}±0.13	4.20^{B}±0.12	4.55^{B}±0.05	0.95^{B}±0.01	1.93^{B}±0.02
Licking	Present	18.4	11.02^{A}±0.17	4.57^{A}±0.15	4.69^{A}±0.07	0.98^{A}±0.01	1.99^{A}±0.03
	Absent	81.6	13.65^{B}±0.11	6.46^{B}±0.10	5.75^{B}±0.05	1.13^{B}±0.01	2.28^{B}±0.02
Looking up	Seen	42.0	11.82^{A}±0.14	5.13^{A}±0.12	5.01^{A}±0.06	1.03^{A}±0.01	2.09^{A}±0.02
	Not seen	58.0	12.85^{B}±0.13	5.90^{B}±0.12	5.42^{B}±0.05	1.08^{B}±0.01	2.18^{B}±0.02
Milking area							
Temperament score	Docile	41.8	13.09^{A}±0.14	5.99^{A}±0.13	5.17^{A}±0.06	1.11^{A}±0.01	2.24^{A}±0.02
	Slightly restless	31.4	12.48^{B}±0.15	5.25^{B}±0.14	4.93^{B}±0.06	1.05^{B}±0.01	2.13^{B}±0.03
	Restless	12.5	12.18^{C}±0.20	5.49^{BC}±0.18	5.27^{B}±0.08	1.05^{B}±0.02	2.13^{B}±0.03
	Aggressive	8.1	12.52^{C}±0.24	5.75^{C}±0.22	5.39^{B}±0.10	1.09^{C}±0.02	2.21^{C}±0.04
	Nervous	6.3	11.41^{D}±0.26	5.10^{D}±0.23	5.32^{B}±0.10	0.98^{D}±0.02	1.97^{D}±0.04
Elimination behaviour	Defecation	11.7	12.60^{bc}±0.20	5.73^{b} ±0.18	5.32^{b}±0.08	1.06^{ab} ±0.02	2.15^{ab} ±0.03
	Urination	6.4	12.16^{a}±0.26	5.36^{a}±0.23	5.14^{a}±0.10	1.05^{a} ±0.02	2.12^{a} ±0.04
	Both	7.6	12.55^{c}±0.24	5.78^{b} ±0.21	5.30^{b}±0.10	1.08^{b} ±0.02	2.19^{b} ±0.04
	None	74.3	12.04^{ab}±0.11	5.19^{a}±0.10	5.10^{a} ±0.04	1.03^{a} ±0.01	2.08^{a} ±0.02

Values with different superscript in upper case letters in column differ significantly at P<0.01 & in lower case letter at P<0.05

Table 2 Rank correlation between cow behavior at milking and milkability

Parameters	Total daily milk yield	Milk yield/ session	Machine-on time	Average milk flow	Peak milk flow
Orientation	0.348**	0.318**	0.321**	0.290**	0.288**
Movement	0.528**	0.488**	0.507**	0.428**	0.422**
Licking	-0.375**	-0.340**	-0.358**	-0.262**	-0.262**
Looking up	-0.180**	-0.172**	-0.179**	-0.143**	-0.142**
Temperament score	-0.143**	-0.071*	-0.012	-0.155**	-0.153**
Elimination behaviour	0.017	0.045*	0.053*	0.028	0.027

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

with a strong correlation with dominance order, which depends on several factors including their level of production. The effect of cow behaviour on milkability could assess the level of comfort a cow feels during milking, as cows which orient themselves towards the milking area waited for their turn of milking, others trying to escape the situation. Such cows need to be selected for milking in advanced milking parlours, especially where batch milking is done so that milking process is completed comfortably with increased cow comfort and milker comfort.

In the holding area, there were animals which showed licking behavior (18.4 % cows) and looking up behavior (42.0 % cows) while waiting for their turn of milking. These behavioural traits in dairy cows were also found to affect their milkability. The cows which were engaged in licking the fellow animals yielded significantly (P<0.01) lower amount of milk (11.02±0.17 kg) with a poor flow rate (0.98±0.01 kg/min). There was significant negative correlation for animals engaged in frequent licking with the milkability traits (Table 2). Similarly, the cows which had tendency to look frequently with their heads raised also had lower yield and milking rate, which was negatively correlated. Ishiwata et al. (2005) in a similar study reported that some cows are cautious about the surrounding environment and might also be fearful of the surrounding environment including humans and other cows (Hemsworth et al. 2000; Munksgaard et al. 2001). This might be the reason for lower yield in such cows exhibiting licking and looking up behavior. The results of present study were in agreement to the reports of Ishiwata et al. (2005) who found that the cows showing more 'looking up' behaviors took longer time

to enter the milking parlor and had lower milk yields irrespective of lactation period.

The differences in milk yield due to temperament score in dairy cows was highly significant (P<0.01) (Table 1). A significant negative correlation was found for the temperament score of cows with milkability (Table 2). No definite trend due to elimination behaviour was seen on milkability of cows. The observations were in agreement to Kumar et al. (2019) who reported significantly (P<0.01) higher milk yield, milking durations and milk flow rate for docile cows compared to nervous cows. Similar findings were reported by Bagnato et al. (2007), Gergovska et al. (2012) and Chauhan et al. (2013). Prasad and Jayalaxmi (2014) reported higher milk yield in docile cows (nearly three times more) compared to nervous cows, which has tendency to hold-up milk in stress situation. Jacobsen et al. (2008) reported that temperament traits have major effect on the time needed for servicing the individual animal and therefore they are extremely important on large farms. The machine-on time due to temperament was more (P<0.01) in aggressive cows due to difficult handling and more of milking irregularities, like cluster slips and reattachments. The aggressive cows had significantly (P<0.01) lower average and peak flow rate, which prolonged the machine-on time and also reduced the milk yield. The higher temperament score of dairy cows in such parlours may be due to uneasiness of being milked in batches, non-adjustment in making group entry inside the milking area i.e. on milking platform or unfavourable milking side of double row parlour. Chauhan et al. (2013) also reported higher milk yield in docile cows under similar environment in comparison to aggressive cows due to more let down time and difficult handling. Urination and defecation are frequently observed in milking parlours. The pattern of defecation and urination is also quite variable, as any kind of stress may stimulate this behaviour (Kulinova et al. 2012). In the present study, it has been tried to know the effect of occurrence of this particular behaviour on various milkability traits. Robichaud et al. (2011) reported that frequency of defecation and urination were not correlated with parity, milk production, body weight, days in milk or dry matter intake in dairy cows. Aland et al. (2002) found that most defecation occurred during the hours when the animals were most active; that is, during milking and feeding. Therefore, it is quite necessary to have a better understanding of this behaviour in dairy cows so that milkability and milk quality are not affected by this behaviour and better management practices could be developed to maintain hygiene in the milking parlour. In a report, it was mentioned that buffaloes before they enter the antechamber of the milking parlor, should be washed to stimulate defecation and urination, and eliminate horn flies. It also promotes body cleanliness, thermal comfort and reduces stress (de Gusmao Couto 2016).

Conclusions

The results revealed that relocating parlour in lactating cows may cause stress in animals with a sharp decline in their milk yield. The animals although have tendency to adapt in a new system, shifting them in the midst of lactation may cause production losses and previous production levels could not be attained by the shifted cows. It may therefore be suggested that any such change made at a livestock farm should get the animals acquainted before the lactation commences which could possibly minimize their production losses. The cow behaviour post-adaptaion in milking parlours (herringbone type) may serve as potent tool to select and split large herd suitable for batch milking based on milk yield and milk flow rates.

Acknowledgments

The authors express sincere thanks to the Director, NDRI, Karnal, for providing necessary facilities and funding for carrying out the research work.

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Indian J Dairy Sci 74(6): 516-525
https://doi.org/10.33785/IJDS.2021.v74i06.008

RESEARCH ARTICLE

Adoption of food safety practices in the informal milk processing units of Haryana, India – A value chain approach

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Received: 31 July 2021 / Accepted: 21 November 2021 / Published online: 21 December 2021

Abstract: In this paper, we analysed how the adoption level of food safety practices varies with milk procurement system in the informal milk processing sector and also developed a composite food safety index (FSI) deliberating the interplay of factors and actors. Primary data were collected from value chain actors, *viz*., small and micro dairy processing units (main actors), milk supplier, *i.e.*, dairy farmer and milk vendors (upstream actors) and traders (downstream actors) in Karnal district of Haryana state of India. FSI of two identified milk procurement systems, *viz.*, (i) own collection centres, and (ii) private vendors were compared. Processing units with own collection centres in production catchments have been able to establish better linkages with dairy upstream actors and resulted in better overall FSI (0.66) than those processing units procuring milk from private vendors (with FSI as 0.51). Further, the profitability of the processing unit is positively associated with the FSI, as consumers are willing to pay higher prices for safe milk. There was a need to design efficient milk procurement systems; design and development of efficient milk transport systems and processing technology along with training in post-milking handling for small vendors and processors for better food safety compliance in the milk value chain of the informal sector.

Keywords: Food Safety, Milk, Procurement system, Value Chain

Introduction

The journey of the world's largest milk producing country started from a novel idea of "Operation Flood" way back in 1970. It has transformed India from a milk deficient nation to surplus. India advanced in milk production and surpassed the rest of the world, with 18% share in global production. Milk is recognised as a complete food for human beings, as it provides essential nutrients (energy, proteins, vitamins and minerals) in a significant amount than other foods of animal origin (Pandey and Voskuil, 2011). Milk production, in India, has attained a compounded annual growth rate (CAGR) of 4.5% over the last 20 years, much higher than the world (FICCI, 2020). However, consumer concern and awareness about milk quality and safety in recent years – attracted attention of the dairy industry (Malathi, 2017). Food safety is one of the essential components of food security (Carlsson et al. 2005; Goldberg and Roosen, 2007; Marette et al. 2008; FAO, 2009; David and David, 2017).

Milk is a perishable commodity, which deteriorates easily if not handled properly, and affects the quality of the dairy products (Murphy et al. 2016). Milk can be contaminated by microorganisms at any stage in the milk value chain from production to consumption and it can lead to serious food borne diseases (Bereda et al. 2012). In developing countries, compliance with food safety measures is found to be elusive along the value chain (Janssen and Swinnen , 2019; Gupta et al. 2014; Handschuch et al. 2013). Food Safety and Standards Authority of India (FSSAI), a regulatory agency – enforcing food safety standards in India, in a survey found that nearly 70% of fluid milk samples did not conform to standards (Centre for Science and Environment, 2012). Demand for safe and quality milk products (Kohli and Garg, 2015), higher income (Gandhi and Zhou, 2013), and increasing integration with the global economy (FAO, 2013), favours food safety. Modernised retailing and speedy urbanisation (Reardon

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and Timme, 2014), in India is forcing the Government for better compliance with food safety measures.

Food safety compliances enhance the competitiveness of small dairy holders (USDA, 2017), and win consumer confidence in the integrity of the value chain (John, 2012; Thakur et al. 2021). To improve the food safety aspect in developing countries, major emphasis has been given to the production activities (Unnevehr, 2014). Studies in Indian dairy sector on food safety issues by Kumar et al. (2011, 2017, 2020) identified its drivers at farm level, impacts on performance (milk yield, profitability) of dairy farms suggest an integrated approach, which ensures food safety starting from production till consumption point.

In India, more than 70% of milk marketable surplus sold through informal channel–consists of vendors, small scale processing units, local traders, where quality is a big concern (Chellappa and Haran, 2018). With multiple actors in the value chain, few questions arise: (i) what is the extent of food safety measures (FSM) adopted by dairy value chain actors? (ii) whether selection of varying procurement systems by processing units – make any difference in composite food safety index? However, past studies did not cover the perspective of food safety measures in the entire milk value chain. In this backdrop, it became essential to address the food safety issues of milk value chains of the informal sector with special emphasis on post-production aspects. Specially, this paper develops deep into the status of food safety practices (FSP) adoption by actors/stakeholders in different stages of (i) own procurement, and (ii) private vendor chain on the basis of procurement pattern by processing units. In the milieu, a composite food safety index was developed and compared for both the chains at different stages as well as for the whole chain. The purpose is to find out which of the chain(s) are complying with safety practices effectively. The knowledge emanating from this study would provide deep insights into implementation of food safety practices and would be helpful to policymakers and planners to formulate policies and programmes to promote safe production of milk and milk products.

Data and Methodology

The study was carried out in Haryana state of India pertaining to year, 2019. Haryana has the highest average milk productivity of Buffalo (8.39 kg/day) and ranks 2nd in terms of per capita milk availability (930 g/day) in the country (NDDB, 2017). Moreover, the share of income earned by household from livestock was (18%) higher as compared to the national average (11%) (Singh et al. 2017). In this study, the Karnal district of Haryana was purposively selected (Fig.1) for primary data collection.

The study region is having a well-established mix of formal and informal milk processing units and earmarked as cluster for milk processing by Government of Haryana (GoH, 2019). For assessing compliance of milk safety practices in the informal processing sector, 27 small dairy processing units were randomly selected using random walk method. These processing units have established backward linkages with farmers and vendors for getting uninterrupted supply of milk. To trace level of food safety measures adopted in upstream actors: 50 milk supplier (dairy farmer and milk vendors) and downstream actors: 20 traders, were selected for detailed data collection. Overall, a sample of size 97 actors involved in milk value chain was studied to develop the composite food safety index for milk and milk products. The primary data collected cover a wide range of information regarding stakeholder's participation in the milk value chain, various food safety practices adopted along the chain. Information on socio-economic profile of stakeholder such as age, education, experience in dairying, *etc.*, were also collected. The key variables used in study included food safety index, value chain actors, value added products, value chain, profit, education of owners of processing units, age, experience in milk processing. The details of conceptual framework and estimation procedure are given as below.

Adoption of food safety practices across the informal milk value chain

To assess the status of FSP adopted by different stakeholders in the milk value chain, potential practices were first identified through literature review followed by consultation with the experts. About 70 scientific practices from literature (Burke et al. 2018; Asselt et al. 2014; FAO, 2013; FSSAI, 2011; NDDB, 2016), which control chemical or biological loss to milk and milk products along the milk value chain (Griffiths, 2010) were listed for food safety practices. Least important practices were omitted at initial stage and finally 39 practices retained to access the food safety score (Table 1). Further, importance of safety practices was measured on a three-point continuum (NDDB, 2016), *i.e.*, critical practices (maximum score of 8), major practices (score ranged between 5-7) and minor (score of 4 or less) from compliance point of view with the help of experts. Finally, per cent adoption of food safety practices at each stage by value chain actors was assessed.

These selected practices were assessed value chain wise and also unit operations wise, *viz.*, i) milking and post milking ii) milk transport and reception iii) processing operations and marketing (Table 1). A norm for compliance was 85 % for major and 70 % for minor safety practices (NDDB, 2016).

The sources of contamination in raw milk were dairy farm environment, producer's hygiene and instrument used (Gashaw et al. 2018; Lemma et al. 2018; Giffel et al. 2010). Milking and post-milking includes 9 scientific practices for milking, storage temperature after milking, personal hygiene of dairy producers, washing of utensils, etc., which prevent milk contamination (Tadele et al. 2016). Transportation of milk from dairy farms to the processing plants must ensure that temperature must not go

Fig. 1 District map of Haryana state

Respondent	Sample size
Milk Producers / Vendors	50
Processing units	27
Traders	20
Total	97

Table 1 Safety practices identified and their adoption across value chain

Sr. No	Indicators/ Actors	Critical /Major / Minor	Max. Score (Scientific practices)	Score Obtained		% Adoption (Based on Count method)	
				Own collection	Private vendor	Own collection	Private vendor
A	Milk and post milking handling (Dairy farmers)						
1	Hygiene/ health of the milking personnel	Critical	8	5.6	5.0	70.0	62.5
2	Udder preparation & disinfection procedure	Critical	8	7.0	5.3	87.5	66.2
3	Method of milking	Critical	8	6.4	5.5	80.0	69.1
4	Time taken to store milk after milking	Critical	8	5.4	2.7	67.5	33.8
5	Washing of utensil and milking machines	Major	6	5.4	3.4	90.0	55.9
6	Milking area is clean	Critical	8	5	5.0	83.3	83.3
7	Milk equipment material (Steel, Plastic)	Major	6	4.4	3.8	73.3	62.7
8	Separate milking of animals under treatment	Critical	8	8.0	8.0	100.0	100.0
9	Milk storage is free from outside contamination	Critical	8	8.0	8.0	100.0	100.0
	Total		68	55.2	46.6		
B	Milk transportation (Processing units/Vendors)						
1	Temperature of milk during transportation	Critical	8	6.4	6.0	80.0	75.0
2	Time taken for transporting raw milk	Critical	8	5.6	4.0	70.0	50.0

3	Vehicle used for transportation of milk from farm	Critical	8	3	3.0	37.5	37.5
4	Vehicles used only for milk products	Minor	4	2	2.0	50.0	50.0
5	Cleaning of bulk tanks, vehicles, containers	Critical	8	5	5.0	62.5	62.5
6	Milk collectors check the farms condition /status	Major	6	5.6	0.4	93.3	5.9
7	Milk suppliers have any kind of training in raw milk handling	Major	6	0	0.0	0.0	0.0
8	Platform test for milk received from farmers at reception point	Critical	8	4.4	4.0	55.0	50.0
9	Infrastructure of collection unit	Major	6	4.8	4.2	80.0	70.6
	Total		62	36.8	28.6		
C	Processing and marketing (Processing units/traders)						
1	Design of unit provide permit maintenance, clearing & prevent entry of dust , dirt & pest	Critical	8	4.5	5.0	56.3	62.5
2	Separate processing for heat treated milk and milk products	Minor	4	0.3	0.0	7.5	0.0
3	Milk and raw material are inspected at the time of reception for food safety	Critical	8	6.4	4.0	80.0	50.0
4	Time taken for delivering milk to processing unit from farm	Critical	8	5.0	4.0	62.5	50.0
5	Incoming materials are sorted according to the storage requirements	Minor	4	2.6	2.0	65.0	50.0
6	Pasteurization process	Critical	8	5.0	5.0	62.5	62.5
7	Hygienic status of milk tanks, valve, pipeline, packaging machine	Critical	8	2.6	2.0	32.5	25.0
8	Post pasteurization process, milk is cooled to 4 or lower	Critical	8	0.8	0.0	10.0	0.0
9	Requisite time achieved is monitored, recorded while processing	Critical	8	8.0	8.0	100.0	100.0
10	Water used in product preparation	Critical	8	4.2	3.9	52.5	48.5
11	Sorting chemical and other substances	Minor	4	3.0	3.0	75.0	75.0
12	Product are packed	Critical	8	6.4	6.0	80.0	75.0
13	Product storage	Critical	8	6.4	6.0	80.0	75.0
14	Cleaning schedules (Regular/ occasional)	Critical	8	6.0	4.0	75.0	50.0
15	Milk waste management	Major	6	6.0	6.0	100.0	100.0
16	Trained workers for milk processing	Critical	8	5.6	4.0	70.0	50.0
17	Periodic review of health status of workers	Critical	8	0.0	0.0	0.0	0.0
18	Hygiene status of workers	Critical	8	6.0	6.0	75.0	75.0
19	Milk handlers uses suitable apron, gloves, headgear, shoe cover, etc.	Major	6	0.0	0.0	0.0	0.0
20	Vehicles used for product transportation	Major	6	3.3	3.0	55.0	50.0
21	Waste disposal patterns of effluent from processing unit	Major	6	4.0	4.0	66.7	66.7
	Total		148	86.1	75.9		

beyond 5°C (Ali and Fischer, 2002). To maintain temperature and other hygienic conditions, 9 practices ensuring milk safety during transportation and reception of milk were included.

During processing operation and marketing of dairy products, potential source of contamination were: (i) physical like dirt particles, hair, metal partials, *etc.* (Kumar et al. 2018), and (ii) microbiological and food borne pathogens (Kamana et al. 2005). Altogether, 21 practices included on quality and hygiene conditions at processing units, training on food handling, pasteurisation of milk, product storage, refrigeration, packaging, milk transport to outlet and subsequently methods used for disposal of by-products/waste material.

To assign a score to each selected practice, opinions from 50 experts (scientists, veterinarians and food technologists) were ascertained. Processing operations and disposal were given a maximum of 148 scores, while milking and post milking practices assigned 68 score and transport activities (62 score) to out of a total score of 278 assigned to all the practices if followed in the value chain.

Safety index was calculated based on the given formula.

$$\text{Safety index} = \frac{\text{Obtained Score}}{\text{Maximum Obtainable Score}} \text{ X } 100$$

Importance of sub indicator was assessed based on the adoption score. In the case of the dairy processing value chain, processing operation and product disposal share 54% of safety score, followed by milking and post milking (24 %) and transport and reception (22 %). Computing the safety score in the chain and classifying them in to three different adopter groups of high, moderate and low adopter based on 80 percentile score (high) (Gupta et al. 2014), 60 percentile (moderate) and lesser than 60 percentiles as cut-off for safety score with the consultation of experts**.** Statistical comparison of the safety scores of selected indicators among two value chains based on (i) own collection and (ii) vendor system of milk procurement was carried out by using t-test with unequal variances. Hypothesis tested that safety scores under two value chains are same and do not differ significantly. The t-statistic for unequal variances of t-test is given by

$$t = \frac{X_1 - X_2}{\sqrt{\frac{s_1^2}{n_1}} + \sqrt{\frac{s_2^2}{n_2}}}$$

Here, X_1 represents the safety score obtained under different indicators in the first value chain and X_2 represents the safety score of second group indicators. Here, n_1 and n_2, represent the numbers of units and s_1^2 and s_2^2 represent variances of the safety scores of units. Unequal variance t-test compares the average value of the two groups, based on the differences in their variances and must be followed over the student t-test, Mann-Whitney U-test (Ruxton, 2006).

Results and Discussion

Prevailing milk value chain of informal sector

Dairy processing units are the core actors in the value chain and other actors entail in the value chain are producers, milk suppliers/ vendors, processing units, distributors and consumers. These processing units are enshrined for compliance of safety practices by regulating the processing operations on one hand and encouraging upstream actors (milk producers and vendors) and downstream actors (traders and consumers) of the chain. Milk value chains were classified on the basis of milk procurement pattern followed by the processing units in the study area. Two milk value chains of the informal sector: first own collection and second, private vendor chain were identified. Using Vensim-7 simulation software (Shamsuddoha and Nedelea, 2013) (free version), the identified value chain of informal processing units, *viz*., (i) value chain of own collection system (Fig. 2) and (ii) value chain of private vendor system (Fig. 3) was mapped with core actors and basic activity performed by them along the chain.

Milk safety in the value chains depends on the safety maintained at each step (Valleva et al. 2005). Hence, prime responsibility of clean and safe milk production lies at farm level. In the first channel, farmers supplied milk to the collection centres of processing units and in the second channel milk was sold to the milk vendors. Milk collection centres is the establishment of some processing units in the production catchments for collecting milk from the producers. Collection centres has the facility of cooling the milk, storage and transportation to the processing units. Private vendors' are one of the important actors in the milk value chain, who are involved in collecting milk from the farmers. They are engaged primarily in direct selling of milk to the end customers and also to the informal processing units, if some quantity is left-out.

Small scale processing units are major stakeholders in the informal milk processing sector. Processing unit owners' exhibits control over the value chain, as they set the quality parameters for milk procurement mainly fat (%), Solid-not-fat (SNF), control processing operations, provide information backward in the chain and retailing of the dairy products. Processing units procure milk either from collection centres or supplied by private vendors. The role of traders/distributors in the milk value chain is to ensure supply of milk and milk products to the consumers and act as a link between processing units and final consumers.

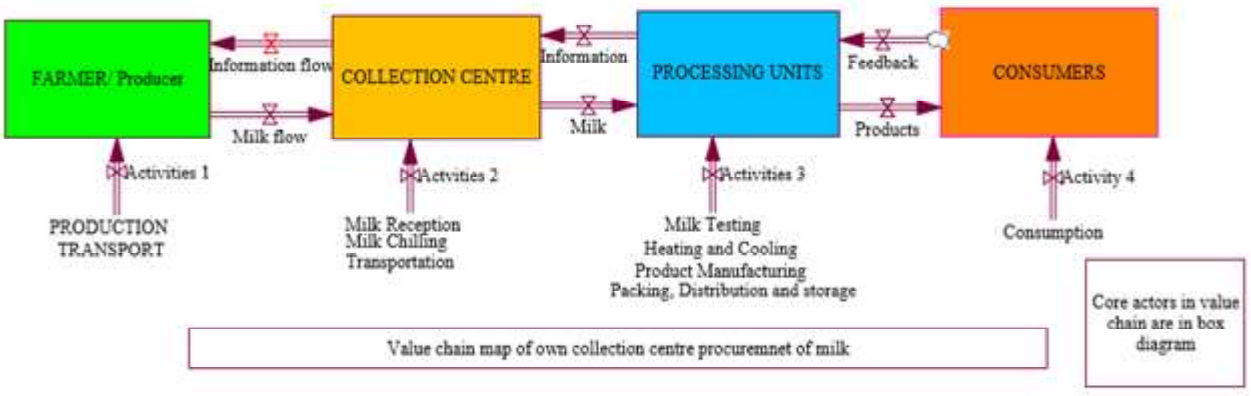

Fig. 2 Value chain of own collection system

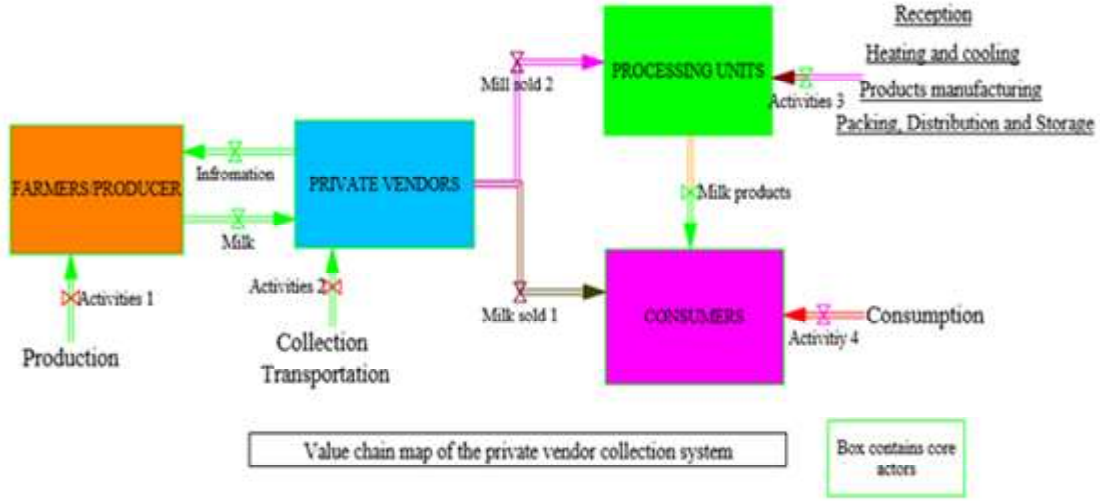

Fig. 3 Value chain of private vendor

Table 2 Food safety index for the milk value chain according to procurement system

Indicators	Overall safety score	Variance			t-value	p-value
	Own collection	Private vendor	Own collection	Private vendor		
Milking and post milking	0.81***	0.69	0.005	0.002	4.96	0.001
Transport activities	0.59***	0.46	0.004	0.001	6.3	0.0002
Processing and Marketing	0.58**	0.51	0.08	0.002	2.46	0.017
Overall Index value	0.66***	0.51	0.003	0.001	5.79	0.0001

*** ($p<0.01$) and ** ($p<0.05$) significance level

Actors involved, activities performed and benefit-cost structure differ with value chain based procurement systems (Thakur et al. 2021). Likewise, adoption of food safety practices varies with the value chain.

Adoption of food safety practices at each stage of milk value chain

Food safety practices found to be adopted at farm level are cleaning of utensils, udders before milking, maintaining personal hygiene, cleaning milk shed, *etc*. Vendors were collecting fresh milk from farmers, washing their milk containers before and after the use. Collection centres of processing units were performing cooling of the milk, storage and transporting milk to processing units by following temperature and time requirements. Personal and vehicle hygiene were maintained at collection centres. Processing units were collecting milk and checking for fat and SNF of milk, adequate storage of milk and milk products.

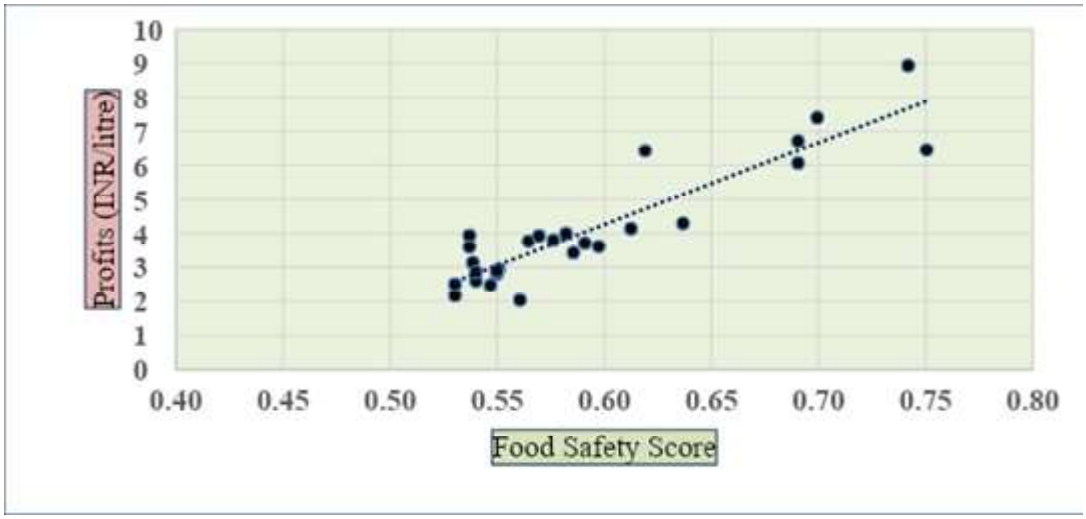

Fig. 4 Food safety index and profits from milk sale

Value chains of milk based on collection centres and private vendors were assessed for the safety practices compliance at each stage/indicator, *viz.,* i) milking and post milking, ii) milk transportation and reception, and iii) processing operations and marketing of milk products. Table 1 reveals that the collection centres value chain has moderate level of safety index values (obtained score of 55.2 out of obtainable score of 68) and private vendor chain was identified as low in safety practice adoption (obtained score of 46.6 out of obtainable score of 68). Transportation of milk with low score (28.6 out of 62) in case of private vendors, is really a matter of concern, as it is difficult to maintain the temperature requirements during summer season, due to non-availability of customised milk cooling van for small milk suppliers/vendors. Hence, delivery box/ milk cooling vans or vehicles, which could maintain temperature, need to be designed for milk suppliers (*Dudhia*). Operations of processing units fall under low levels of safety adoption, with scores obtained of 86.1 and 75.9 out of 148, in collection centres and private vendors system, respectively (Table 1). This is because of the fact that the critical activities like maintaining proper pasteurisation temperature, use of portable water for product preparation, storage of products according to temperature required, and waste disposal mechanism have not been properly followed by these processing units. Specialised training and skill development on adoption of proper processing protocols need to be imparted.

Food safety index

Safety scores obtained for the different safety indicators and overall index for the value chains in the study possess different values. The hypothesis herein is that there is no significant difference in the safety score obtained in the value chain of own collection centres and private vendors chain and it was tested by using t-test for unequal variances. Results obtained from the t-test (Table 2) reject the hypothesis and confirm that significant differences exist between the overall indexes of the two value chains.

All the indicators in the index were tested individually among two chains. It has been found that indicators of safety scores as well as overall index values in the own value chain differs significantly from the private vendor chain.

Cut-off scores for classifying the value chains as high, moderate and low adopters were 0.8 (80 %), 0.6-0.8 (60-80%) and below 0.6 (<0.60 %), respectively.

Food safety index (FSI) calculated for the milk value chain of own collection centres (0.66) was higher as compared to FSI of private vendors value chain in the study area (Table 2). Value chain of the own collection centres comprises of the farmers who supply milk to the collection centres of processing units. Processing units, who have established collection centres, check the quality of milk (SNF, Fat) at initial stages of the value chains, which meets certain pre-requisites of safety practices. Collection centres has the advantage of milk cooling facility, which reduces the chance of milk spoilage and ensure safe milk transport to the next stage, *i.e.*, processing units. FSI value in case of private vendors' value chain was 0.51 (Table 2). This value chain comprises farmers and private vendors who collect the milk from the farmers and deliver it to the processing units. There is no provision of milk quality check in the initial stages of the chain, although these processing units assess the milk quality at later stage through lactometer test, organoleptic test and checking milk fat and SNF by Gerber test. FSI of own collection centre significantly differ than private vendor chain. To check the statistical difference of safety index values among the identified chains, t-test statistics was applied. FSI of own collection centre was significantly higher than private vendor chain for all selected indicators. This may be on account of unified management– enable strong linkages with dairy farmers, in case of own collection centre value chain, besides underlying factors, *viz.*, processors

technical knowledge, availability of milk testing and cooling facilities, and timely transport. These collection centres impart knowledge to farmers on food safety issues and act as a bridge between dairy farmers and processing units. Major reason for lower safety index values in private chain was lack of control of processing units over the value chain actors. Private vendors deliver milk directly to the processing units; and hence, no interaction between producers and processors regarding safety issues and milk quality. Milk vendors hardly follow the temperature and time requirement from milk safety standpoint. During survey, it was observed that educated and experienced processors taking care of food safety norms. Further noticed that processing units procuring higher volume of milk than their capacity reluctant towards food safety norms. The training of manpower and actors and adoption of proven processing technology could contribute improvement of FSI in dairy value chain.

Food safety index at farm level

It has been found that most of the safety practices at the milk producer's level have been implemented, the results collaborate with findings of Kamana et al. (2017) and Kumar et al. (2011). However, the intensity of adoption has been varying among the producers associated with different chains. Food safety practices score for milking and post milking practices was 0.81 (Table 2) for the producers who deliver milk directly either to processing units or their collection centres and in the case of private vendor procurement chain, it was 0.69. This means that 81 per cent of the safety compliance has been followed in collection centres and 69% in the private vendor chain. The major difference in the safety scores were attributed to the storage and cooling facility made available at collection centres in the production catchment itself. Use of plastic cans for milk collection and storage has been more common in practice for those who supply milk to the private vendors. Alternatively, metal cans and containers were more used by the producers in own collection centre chain, which reduces the chance of milk contamination (Gashaw et al. 2018).

Food safety index of milk transport and reception

Critical safety practices under the transportation indicator were rapid cooling of milk, and temperature of milk maintained throughout the transport, which can reduce the microbiological activity, especially bacteria (Gashaw et al. 2018). Rising milk temperature during transportation reduce the milk quality hence rapid cooling after production must be followed to enhance the shelf life of the milk. It has been found that in the chain where milk is delivered to the collection centres which complies with critical safety practices of cooling and transporting milk to the processing units under the temperature requirements (<5°C) have lesser chances of bacterial growth (Valeeva et al. 2005). Milk value chain of private vendors do not comply with safety regulation of temperature (4-5 °C) (ILRI, 2007) as they do not possess any facility for milk chilling and it takes 5-6 hours for them to reach the processing units which reduces the milk quality (Lemma et al. 2018). Safety score assessed for the transportation activities in collection centres value chain and private vendors chain were 0.59 and 0.46, respectively (Table 2). Major practices like use of refrigerated vehicles for transportation of milk, insulated bulk containers were not in practice in both the chain and reason for the above was lesser volume of milk handling. Milk quality were satisfactory at farm level but deteriorates most during the transportation from farm to processing units (Kumar et al. 2011).

Food safety index at processing operations and marketing

FSI values for the processing units were 0.58 (58 % adoption) and 0.51 (51% adoption) for value chain of direct/own collection and private vendors, respectively (Table 2). Critical activities identified in the processing units were to perform the platform test for milk (Organoleptic, lactometer, and alcohol test), operation of cooling, pasteurisation, and use of portable water for products, packaging and storage temperature of different products. Processing units were found to comply with certain critical activities but safety norms like pasteurisation temperature (72 °C, 15 seconds) were not monitored in the most of the units. Use of portable water for product preparation were found more in case of owners who are associated with own collection centres. Storage of different dairy products requires different temperatures and dairy processing units hardly found to comply with product specific storage requirements. Major activities identified for processing units were sorting incoming raw materials, trained manpower, unit infrastructure, waste disposal, water management, personal hygiene, record keeping, temperature requirement, regular cleaning schedules. Safety norms like personal hygiene of worker, water and waste management, trained manpower was found inappropriate. Compliance of safety practices were adopted at the lesser level at the processing units who source milk from private vendors. Procurement method of milk by processing units and raw milk processing are critical safety practices which affect safety at processing unit level (Valeeva et al. 2005). Safety practices score in processing operation mainly depends upon the processor's technical knowledge, technology adopted in the processing, infrastructure of units and hygienic conditions maintained in the units.

Food safety index and profit

Overall, adoption of food safety practices in the value chain is expected to increase the milk quality and finally market prices. Empirical results from the study states that adoption of safety practices is positively and significantly associated with final market prices. Value chains with higher safety index values fetch better prices. Compliance of safety norms at the processing levels are encouraged through premium prices in milk paid by consumers. This establishes the fact that processing units complying with more safety practices (*i.e.,* higher safety index value) leads to higher profits per litre of milk sale (Fig. 4). Hence, it can be said

that the average milk price differs with adoption level of food safety practices.

Conclusions

Food safety practices adopted in the milk value chain have been assessed considering two main routes of milk procurement of informal dairy processing units, *viz.*, (i) collection centres, (ii) private vendors. The performance of the processing unit is positively associated with the food safety index (FSI), as consumers are willing to pay higher prices for safe milk. Value chain of own collection centre was found superior in terms of food safety compliance indicating that involvement of one more actor (vendor) has an implication on food safety score. Better the linkages of informal dairy processing units with upstream actors (dairy farmers), better is the food safety compliance.

Hence, institutions like ICAR-National Dairy Research Institute, State Agricultural Universities and other line departments need to take up regular training programmes in post-milking handling. Further, smart milk procurement and transport systems need to be designed and developed for small vendors and processors for food safety compliance in informal milk value chain.

Acknowledgements

The authors acknowledge the help received from project staff under ICAR-NIAP project on Policy Imperatives for Promoting Value Chains of Agricultural Commodities in India during data collection.

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https://doi.org/10.33785/IJDS.2021.v74i06.009

RESEARCH ARTICLE

Economic sustainability analysis of Gaushalas in selected districts of Telangana state

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Received: 06 April 2021 / Accepted: 01 October 2021 / Published online: 21 December 2021

Abstract: In India, with the increasing cattle population the number of stray cattle has also been increasing. While indigenous cattle constitute a major portion of the total cattle population their proportion has been showing a declining trend. Gaushalas can serve as an alternative for housing the increasing stray cattle and also for preserving the indigenous germplasm. However, Gaushalas are functioning with insufficient funds mostly from donations and government grant. Hence, in this study, an attempt was made to undertake economic sustainability analysis of Gaushalas by covering 14 Gaushalas in select districts of Telangana state. The data was collected from Gaushalas for the period 2014 to 2019 using a semi structured interview schedule. It was found that the major source of income to Gaushalas was from donations (82.5 per cent) followed by income from sale of milk and milk products (12.9 per cent). The expenses of the Gaushalas mainly included feed and fodder cost (82.5 per cent) followed by veterinary and miscellaneous expenditure (6.7 per cent). The overall net income for all the Gaushalas was negative. The returns over variable cost were positive for almost all the Gaushalas indicating that the Gaushalas were able to meet their immediate expenses in the short run. Economic Sustainability Index (ESI) was computed which ranges from 0 to 1 using 5 indicators *viz*., net income per animal, returns over variable cost, self-sufficiency, dependency and percentage of productive animals. The values of ESI of the Gaushalas ranged from 0.302 to 0.457. Among14 Gaushalas 2 Gaushalas fell under low economic sustainability and rest 12 Gaushalas were under medium economic sustainability category. A significant positive relationship was found between income diversification and sustainability of the Gaushalas (r=0.73, P<0.01). So, there is a need to diversify income sources such as income from sale of milk, milk products and other by-products.

Keywords: Breed conservation, Cow slaughter, Indigenous cattle, Stray cattle, Unproductive cattle

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Introduction

India is blessed with rich livestock diversity. Livestock sector plays a major role in Indian agrarian economy. Livestock sector accounts for 4.2 per cent of the total Gross Value Added (GVA) and 28.6 per cent of GVA for agriculture sector (GoI, 2020). About 62 percent of the marginal farmers were associated with livestock and 17 per cent of their total income came from the livestock sector (Das et al. 2020). The total cattle population in India has reached to 192.49 million in the year 2019 from 190.90 million in 2012 (GoI, 2012 & 2019). The major portion of cattle in India constitutes indigenous cattle up to 73.8 per cent of the total population as against 26.2 per cent of crossbred cattle. While the population of crossbred increased by 26.9 per cent from 2012 to 2019 the indigenous cattle population fell by 6.0 per cent. (GoI, 2012 & 2019). The decreasing trend of indigenous population is a matter of serious concern as this may lead to loss of diverse genetic base of India (Yadav and Vij 2010; Mandi et al. 2018). The major reason for this problem is that farmers are showing preference to crossbred cattle over native cattle as the milk yield of crossbred cattle is more when compared to that of indigenous. When the milk yield of cattle decreases after 7th or 8th lactation farmers abandon them. Thus, these unproductive and old cattle with no source of feed start wandering on farms and destroying crops. In urban areas, stray cattle are usually found eating plastic and other garbage which eventually leads to severe health problems or even death. These stray cattle also pose a threat to humans as they usually roam on highways and other roads leading to accidents.

With further ban on slaughter of cow, the stray cattle population has been increasing. At present there are about 52,87,767 stray cattle in India and 36,366 stray cattle in Telangana state (GoI, 2019). With strict cultural and government restriction against slaughtering of cattle in almost all parts of India it is hard to control their population (Bijla and Singh, 2019). Sheltering stray cattle in Gaushalas can provide an alternative. Gaushalas in some states along with sheltering cattle are also taking up breeding activities to preserve the indigenous germplasm. Efficient management of Gaushalas is necessary as these institutions mostly depend on funds received from donations and government grants. However, Gaushalas face a number of issues like lack of funds, irregular support from government, lack of infrastructure, less space etc. (Bijla and Singh 2019). Hence, it is important to know the sustainability level of these institutes. Thus, in this study an attempt was made to assess the economic sustainability of Gaushalas in Telangana state.

Materials and Methods

Study area and sampling

Telangana is a state situated on the high Deccan Plateau, on the south-central part of the Indian Peninsula. It is India's eleventh-largest state and twelfth-most populous state with a total area of 112,077 km^2 and 35,193,978 residents (Government of Telangana, 2015). The erstwhile state was split from the north-west portion of Andhra Pradesh on 2 June 2014 as the newly created 29th state with Hyderabad as its historic permanent capital. Telangana is largely rural, with roughly two-fifths of the state's population classified as urban. Hyderabad accounts for more than half of those in urban areas.

Telangana is gifted with huge livestock resources. It has a cattle population of 4.23 million head which consists of 0.61 million crossbred cattle (14.43 per cent) and 3.62 million indigenous cattle (85.56 per cent) (GoI, 2019). Cattle in Telangana are protected under the act "The Telangana Prohibition of Cow Slaughter and Animal Preservation Act, 1977" which prohibits against slaughter of animals without certificate from competent authority (Government of Telangana, 2016). Since not many studies could be found regarding Gaushalas in Telangana this study can help understand their functioning from economic perspective.

The required data were collected from a sample of 14 Gaushalas located in southern Telangana spread across 10 districts. A list of sample Gaushalas selected for the study along with their respective cattle population is presented in Table 1. The data regarding number of animals, their composition, funds generated by Gaushalas through donations, government grants, miscellaneous income and expenditure were collected from the records maintained by Gaushalas. Primary data for the study was collected on production and prices of milk and other products, expenditure incurred on green fodder, dry fodder, concentrates, labor and salaries of employees, and veterinary expenses using interview schedules from response persons in each of the selected Gaushala. The collected data were tabulated for five consecutive years from 2015 to 2019 and average value was taken for analysis.

Analytical techniques

Ratio analysis

Ratio analysis was attempted to analyze the economics of Gaushalas. The following ratios were worked out

$$\text{Operating Ratio} = \frac{\text{Total operating expenses}}{\text{Gross Income}}$$

$$\text{Fixed Ratio} = \frac{\text{Total fixed expenses}}{\text{Gross Income}}$$

$$\text{Gross Ratio} = \frac{\text{Total expenses}}{\text{Gross Income}}$$

Table 1 Distribution of cattle population across Gaushalas

S. No	Gaushala	Indigenous cattle	Crossbred cattle	Buffalo	Total cattle population
1	Mahbubnagar	147	0	0	147
2	Gadwal	150	0	0	150
3	Nagarkurnool	350	0	0	350
4	Narayanpet	105	0	0	105
5	Wanaparthy	96	15	0	113
6	Medchal	92	0	0	92
7	Ranga reddy-1	1050	0	0	1050
8	Ranga reddy-2	83	4	0	87
9	Ranga reddy-3	256	0	0	256
10	Hyderabad-1	500	100	0	600
11	Hyderabad-2	4280	202	222	4704
12	Hyderabad-3	50	2	0	52
13	Bhuvanagiri	383	0	0	383
14	Medak	1398	380	0	1778

Income diversification index

Herfindahl-Hirschman Index (HHI) was computed to know the extent of income diversification of Gaushalas (Rhoades, 1993). HHI can be defined as the sum of the squares of the proportions of different sources of income. The degree of diversification can be measured using *HHI* as follows

$$HHI = C^2_1+C^2_2+C^2_3+C^2_4+C^2_5+C^2_6+C^2_7+C^2_8+C^2_9$$

Where,

$C_1, C_2, C_3 \ldots\ldots\ldots\ldots C_9$ = Proportion of different sources of income to total income of Gaushalas

After calculation of HHI, Herfindahl Diversification Index (HDI) was calculated as:

$$HDI = 1 - HHI$$

Higher HDI value indicates higher the income diversification of the Gaushalas. Higher the income diversification of Gaushalas indicates higher the sustainability.

Economic sustainability of Gaushalas

The methodology of assessing the Economic Sustainability Index (ESI) was developed by Singh et al. (2019). In this study, the same methodology was followed for developing ESI.

i. Net income per animal:

Net income was obtained by deducting total expenses from gross income then dividing it by the number of cows in Gaushala. It has a positive effect on sustainability of Gaushalas. The Standard Animal Units were worked out as suggested by Sirohi et al. (2019).

$$\text{Net Income} = \frac{\text{Gross Returns} - \text{Total cost}}{\text{Standard Animal Units}}$$

ii. Returns over variable cost

If this indicator comes out to be positive, then it shows that particular Gaushala is able to meet its variable cost.

$$\text{Returns over variable cost} = \frac{\text{Gross Returns} - \text{Variable Cost}}{\text{Standard Animal Units}}$$

iii. Self-sufficiency/ Autonomy

Proportion of income obtained from the sales of Gaushala products. It includes the income sources other than obtained from government grants and donations.

iv. Dependency

Dependency is calculated by computing the contribution of donations and government grants. For a Gaushala to be sustainable, this indicator should be less.

v. Percentage of productive animals

The productive animals include in-milk and pregnant cows, in-milk and non-pregnant cows, pregnant heifers and service bulls. It has a positive effect on sustainability of Gaushalas.

The data normalization was done to bring all the indicators to a common scale using Min-max technique.

$$I_i = \frac{X_i - MinX_i}{MaxX_i - MinX_i} \quad (1)$$

$$I_i = \frac{MaxX_i - X_i}{MaxX_i - MinX_i} \quad (2)$$

where X_i = Value of i^{th} indicator

$i = 1, 2, 3 \ldots\ldots n$ indicators

Differential weights were assigned to indicators following Singh et al. (2019) which was worked out based on expert opinions. The aggregate *ESI* was calculated as follows

$$ESI = \frac{\sum W_i I_i}{\sum W_i}$$

where

I_i = Normalized value of i^{th} indicator

W_i = Weight given to each indicator from experts

n = Number of indicators

ESI value varies from 0 to 1. The Gaushalas were grouped into three categories based on their value of ESI, *i.e.* Gaushalas with ESI <0.33 were considered as less sustainable, 0.33 <ESI < 0.66 were considered as moderately sustainable, while Gaushalas with ESI > 0.66 were considered as highly sustainable.

Results and Discussion

Cattle population trends in Gaushalas

Analysis of data regarding inflow and outflow of cattle in Gaushalas for the past 5 years showed that there was a continuous rise in inflow and outflow of cattle over the years (Fig.1) except in the year 2019. The rate of inflow was higher than the rate of outflow over the years. In 2019, both the inflow and outflow were lesser as data was collected till October, 2019. The composition of total cattle population in all the Gaushalas is shown in Fig. 2.

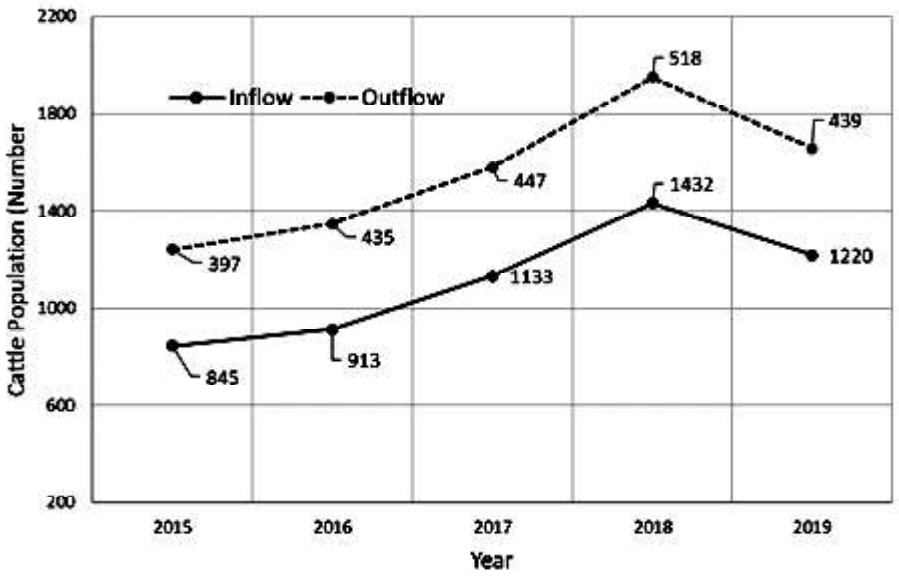

Fig. 1 Trends in inflow and outflow of cattle in Gaushalas

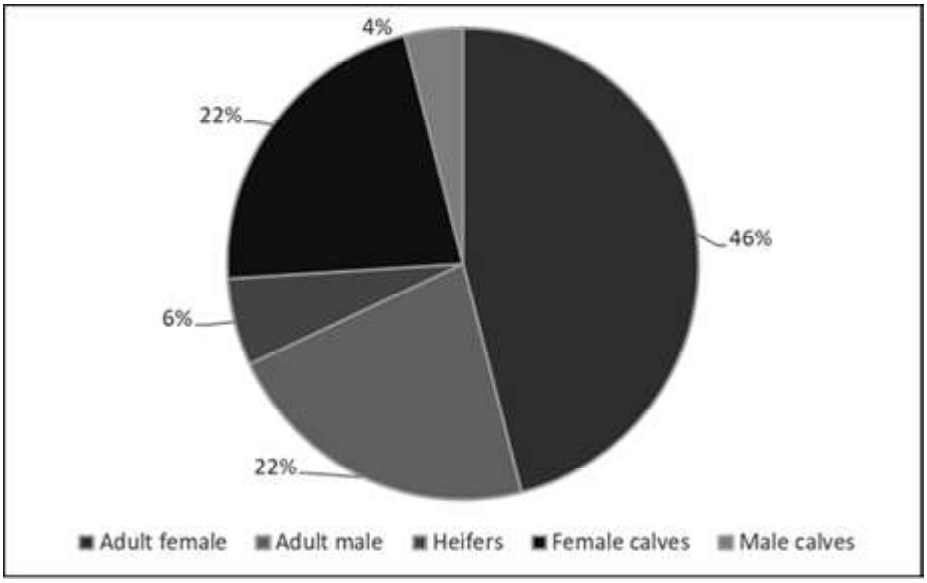

Fig. 2 Age-sex composition of cattle population in Gaushalas

Adult females constitute major portion of total cattle population (46 per cent) followed by heifers (22 per cent).

Income and expenses of Gaushalas

The percentage share of income and expenditure of Gaushalas is shown in Fig. 3 and Fig. 4, respectively. It is seen that the major source of income to Gaushalas was from donations (82.46 per cent). Similar results were reported by Bijla and Singh (2019) in their economic study of Gaushalas in Haryana. It is observed that the monetary support from the Government or state animal husbandry departments was not extended to Gaushalas unlike other states like Karnataka or Haryana where monetary support is provided to registered Gaushalas annually. After donations, income from sale of milk and milk products, compost, cow dung, urine, phenyl and panchagavya contributed to a major portion of income of the Gaushalas (12.93 per cent). High dependence of Gaushalas on donations represented weak sustainability. Miscellaneous income which mainly constitutes money spent personally by owner of Gaushalas and income received from sale of grains, dead animals, sale of scrap, interest on deposits, tax rebate constituted the rest of income (4.59 per cent).

On the other hand, the expenses of Gaushalas mainly included feed and fodder cost contributing about 82.51 per cent followed by veterinary and miscellaneous expenditure (6.68 per cent). The miscellaneous expenditure includes minor repairs, fuel, festival, kitchen, utility, ropes, gunny bags, stationary, municipal taxes, insurance. Fixed costs namely labour expenses and cost of depreciation of assets constituted 5.78 and 5.01 per cent of the total expenditure respectively.

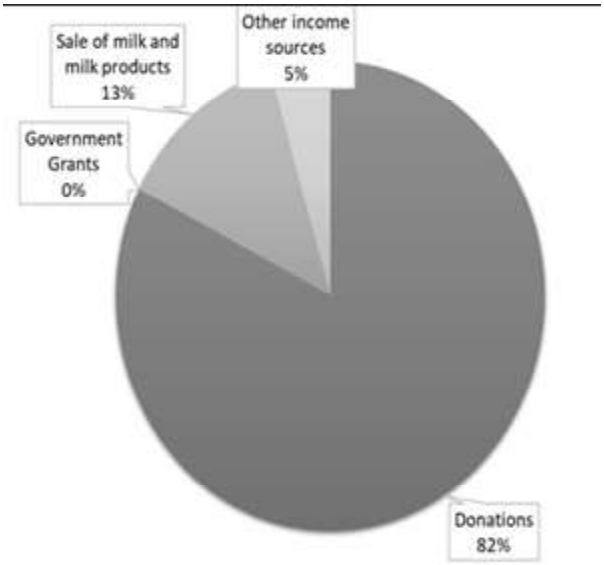

Fig. 3 Income sources of Gaushalas

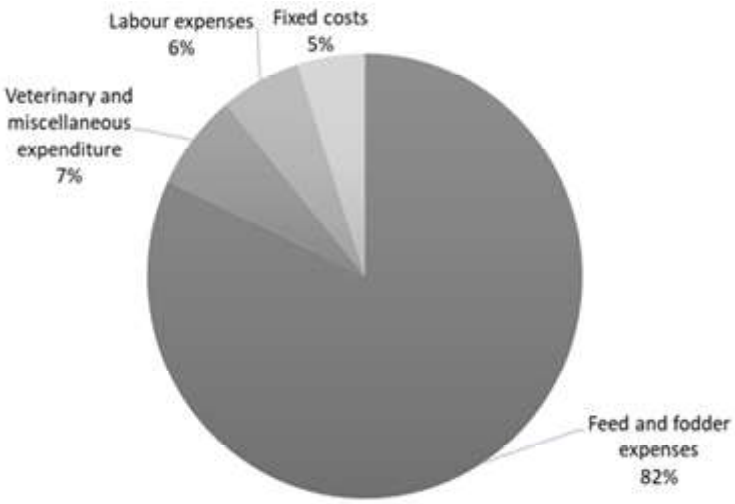

Fig. 4 Expenditure pattern of Gaushalas

Net income across Gaushalas

The average net income of Gaushalas for the period 2015 to 2019 is shown in Table 2. It reveals that most of the Gaushalas had negative net income except two Gaushalas in Gadwal and Hyderabad districts which had a positive net income of ₹ 71,800 and ₹ 12,700 per year respectively. The overall net income for all Gaushalas was negative with ₹ 1.17 lakh per annum. The reason for negative net income was lack of productive animals in Gaushalas. Most of the Gaushalas depended on donations and had very poor income generating sources. They also had high fixed expenses like depreciation of buildings and machinery etc. Large herd size was another reason for negative net income. Some Gaushalas management were against the idea of running Gaushalas for profits as their major objective is protecting stray cattle. Cultural significance towards indigenous cattle and negative opinion towards crossbred cattle was yet another reason for low productivity of Gaushalas. One Gaushala in Medak District had the lowest net income (negative ₹ 81.26 lakh per annum). The reasons for this again were low income, low level of income diversification and large herd size.

Returns over variable cost

When the returns are calculated only for variable costs, positive returns were obtained for most of the Gaushalas. Table 2 shows that returns over variable cost for all the Gaushalas. The positive returns over variable cost indicates that Gaushalas were able to meet their immediate expenditure requirements in the short run. Of all the Gaushalas Hyderabad-2 had the highest returns over variable cost (₹ 42,03,750/year). These high returns could be attributed to large donations received by the Gaushala. Gaushalas in Narayanpet, Wanaparthy, Medchal and Hyderabad had zero returns to variable costs. This indicates that these Gaushalas were only able to meet their variable expenses and were on their breakeven point. They were located mostly in rural areas and outskirts of city making it hard to find donations.

Ratio analysis

Ratio analysis is an important tool to know about the profitability of the Gaushalas and to understand other financial implications (Table 3). These ratio measures indicate what proportion of gross income is spent for meeting different types of expenditure. Gross ratio values less than one indicate efficient functioning of enterprise. It was found that gross ratio for all the Gaushalas is more than one which indicates that Gaushalas were not performing efficiently i.e. they were unable to cover their total expenses with given gross income. Lower fixed ratio ranging from 0.02 to 0.15 (overall ratio 0.06) indicates that most part of the fixed expenses could be covered by prevailing income sources. Operating ratio is the most important ratio analysis which indicates the ability of Gaushala to meet immediate financial requirements in short run. The overall operating ratio of 0.98 indicates that 98 per cent of their income was spent covering only operating expenses. This shows that most of the Gaushalas in Telangana are not financially efficient.

Income Diversification Index of Gaushalas

HDI values range from 0 to 1, zero being the lowest diversity and 1 indicating the maximum diversity. The HDI values of Gaushalas are presented in Table 4. Gaushalas in Ranga Reddy, Hyderabad and Bhuvanagiri were found to have the lowest HDI value of 0 indicating no diversity of income at all. The reason for their no diversity is because these Gaushalas were totally dependent on public donations and did not manage to have income source other than donations. The total dependence of Gaushalas on public donations makes them economically vulnerable in the long

Table 2 Net income and returns over variable cost across Gaushalas

S. No	Gaushala	Net income (₹ 10,000 /year)	Returns over variable cost (₹ 10,000 /year)
1	Mahbubnagar	-9.00	6
2	Gadwal	7.18	20.8
3	Nagarkurnool	-2.94	4.3
4	Narayanpet	-3.66	0
5	Wanaparthy	-3.69	0
6	Medchal	-4.80	0
7	Ranga reddy-1	-20.44	50.4
8	Ranga reddy-2	-14.40	12.8
9	Ranga reddy-3	-2.03	8.4
10	Hyderabad-1	-15.45	40.8
11	Hyderabad-2	1.27	420.375
12	Hyderabad-3	-8.13	0
13	Bhuvanagiri	-7.29	10.56
14	Medak	-81.26	105.2
	Over all	-11.76	48.55

Table 3 Financial ratios of Gaushalas

S. No	Gaushala	Fixed ratio	Operating ratio	Gross ratio
1	Mahbubnagar	0.09	0.96	1.06
2	Gadwal	0.15	0.77	0.92
3	Nagarkurnool	0.04	1.23	1.26
4	Narayanpet	0.03	1.00	1.03
5	Wanaparthy	0.02	1.00	1.02
6	Medchal	0.02	1.00	1.02
7	Ranga reddy-1	0.03	0.98	1.01
8	Ranga reddy-2	0.10	0.95	1.05
9	Ranga reddy-3	0.02	0.99	1.00
10	Hyderabad-1	0.07	0.95	1.02
11	Hyderabad-2	0.08	0.92	1.00
12	Hyderabad-3	0.11	1.00	1.11
13	Bhuvanagiri	0.02	0.99	1.01
14	Medak	0.04	0.98	1.02
	Over all	0.06	0.98	1.04

run when the situations are not congenial. Maximum diversity was found in the case of Gaushala from Ranga Reddy (0.596) which received 57.27 percent of income from sources other than donations followed by Gaushala from Narayanpet (0.585) which received 95.7 per cent of income from sources such as selling of milk, milk products, dung, and other miscellaneous sources. Though Narayanpet Gaushala received 95.7 per cent of its income from sources other than donations its HDI value is less because it received little income from donations and was more dependent on products produced within Gaushala making it vulnerable to production irregularities. On an average HDI of Gaushalas in Telangana was 0.262 indicating very low independence. A significant positive correlation was found between income diversification and sustainability of the Gaushalas (r=0.73, P<0.01). Similar findings were also reported by Bijla et al. (2019).

Economic sustainability index of Gaushalas

ESI values of all Gaushalas along with their respective values of Net Income per (Standard Animal Unit) SAU, returns over variable cost per SAU, autonomy, dependency and percentage productive animals are indicated in Table 4. The values of ESI for the Gaushalas ranged from 0.302 for Hyderabad-2 Gaushala to 0.47 for Hyderabad-3. Two Gaushalas, one from Hyderabad (0.30) and another from Ranga Reddy (0.32) fell under low ESI category (i.e. ESI < 0.33) and rest 12 Gaushalas under medium ESI category (0.33 < ESI <0.66). None of the Gaushalas had ESI > 0.66.

Conclusions

At present Gaushala is a new area of social concern with the increasing restrictions on slaughtering of cattle. The major source

Table 4 Income diversity index and Economic sustainability index values of Gaushalas

Name of the Gaushala	HDI Values	Net income (₹ /year/ animal)	Returns over variable cost (₹ / year/ animal)	Autonomy	Dependency	Proportion of productive animals (%)	ESI Value	Rank based on ESI
Medchal	0.445	-631.03	0	33.38	66.62	21.74	0.457	1
Narayanpet	0.585	-369.51	0	45.19	54.81	23.81	0.455	2
Wanaparthy	0.449	-390.44	0	26.47	73.53	31.86	0.431	3
Gadwal	0.391	604.47	1750.25	10.89	89.11	4.67	0.429	4
Mahbubnagar	0.225	-744.77	496.52	13.31	86.69	17.01	0.417	5
Ranga reddy- 2	0.596	-2341.26	2080.62	11.68	88.32	36.78	0.409	6
Medak	0.101	-450.46	583.14	34.87	65.13	19.57	0.378	7
Hyderabad-3	0.101	-2008.90	0	5.34	94.66	15.38	0.373	8
Bhuvanagiri	0.000	-271.05	392.87	0	100.00	37.34	0.374	9
Ranga reddy-1	0.000	-263.42	649.49	0	100.00	41.05	0.357	10
Hyderabad-1	0.000	-242.04	639.22	0	100.00	4.00	0.351	11
Nagarkurnool	0.068	-103.69	154.39	0.77	99.23	29.71	0.351	12
Ranga reddy-3	0.334	-85.00	352.41	3.49	96.51	4.69	0.324	13
Hyderabad-2	0.018	2.69	892.39	0.82	99.18	1.98	0.302	14

of income to Gaushalas was from donations followed by income from sale of milk and milk products. The Government should implement special schemes for Gaushalas and provide financial assistance to Gaushalas for their sustainability. The net income was found to be negative for most of the Gaushalas due to higher expenditure on feeding large number of animals and very low milk production. Hence, efforts should be made to provide good quality feed and fodder at reasonable prices which will help lowering the expenses of the Gaushalas. Though Gaushalas were able to meet their immediate expenses in short run they were not able to cover variable and fixed expenses completely. Space is yet another constraint with the increasing stray cattle. Government should provide land and other infrastructure to Gaushalas. A positive relationship between income diversification and economic sustainability of the Gaushalas indicates that there is a need to diversify income sources such as income from sale of milk, milk products and other by-products.

Acknowledgements

The first author thankfully acknowledges ICAR-National Dairy Research Institute, Southern Regional Station, Bengaluru for the scholarship.

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https://doi.org/10.33785/IJDS.2021.v74i06.010

RESEARCH ARTICLE

Analysis of role performance and effectiveness of dairy extension service providers in Karnataka State

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Received: 24 September 2021 / Accepted: 29 September 2021 / Published online: 21 December 2021

Abstract: Dairy extension services delivery system providing access to services and critical inputs plays a cardinal role in adoption of technologies by the farmers for improved livestock productivity. In a pluralistic environment, multiple dairy extension service providers extend various services and inputs to the dairy farmers. In this context, the present study was undertaken to assess the role performance of various dairy extension service providers and their effectiveness in Karnataka State. A total of 400 dairy farmers who were availing the services from different extension service providers were selected as respondents for the study using multi-stage random sampling method. The Department of Animal Husbandry and Veterinary Services (DAH&VS) of the State played a predominant role in providing 'breeding services' (87.25% A.I & 90 P.D), 'preventive services' (vaccination 86%, deworming 89%, and 76% disease surveillance) and 'curative services' (82%). Whereas, the Dairy Co-operative Societies (DCS) played a vital role in extending assured 'marketing services' (94%), 'input services' (fodder seeds 86.50% and feed supplements 89.25%), 'implementation of dairy development schemes' (86%) and provision of 'extension advisory and training services' (84.50%). The effectiveness of service providers as perceived by the dairy farmers as per the weighted mean score indicates that DAH&VS was found more effective in extending 'health care services' (278.67) and 'breeding and reproduction services' (274.26), whereas, DCS were more effective in extending assured 'marketing services' (289.73), providing 'input support services' (278.33), 'implementation dairy development schemes' (274.53) and provision of 'extension advisory and training services' (264.35). The present study concludes that most of the dairy extension service providers played a significant role in extending different dairy extension services and inputs to the farmers. However, concerted efforts need to be made by all the stakeholders for possible convergence among them in reaching out the dairy farmers and providing emphasis on 'dairy extension education services' in addition to 'providing inputs and support services' to the dairy farmers.

Keywords: Animal Husbandry and Veterinary Department, Dairy Farmers, Dairy Extension, Dairy Co-operatives

Introduction

The Indian dairying is characterized as 'production by masses' rather than 'mass production'. Dairy extension services delivery system providing the services and critical inputs which are important to the adoption of technologies by the farmers for improving livestock productivity. The primary dairy extension service providers in Karnataka State are Department of Animal Husbandry and Veterinary Service (DAH&VS), Dairy Cooperative Societies (DCS) of Karnataka Co-operative Milk Producers Federation Ltd (KMF), National Agricultural Research and Education System (NARES) institutions, Non-Governmental Organizations (NGO's) and Private Input agencies/Dairies. The dairy extension delivery systems are expected to addresses three important requirements of farmers which includes; technical services, critical inputs and education and advisory services. It is evident from the existing situation at grassroots level where our extension system primarily focused on first two requirements and whereas, the 'education and advisory services' are grossly neglected (Rao and Natchimuthu, 2015). Due to this lacuna, most of the livestock producers being small and marginal farmers, their capacity to overcome the present challenges and to adopt the latest technologies developed by research institutions is limited. Absence of effective extension machinery for this purpose further adds problem. Though each of the institute has different mandate and approaches in reaching out the farmers, most of the time, its

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efforts were sporadic in nature and lack of collaboration among these different organizations poses a serious hindrance for the development of livestock sector.

Though there are many stakeholders involved, in reality they focused mainly on provision of 'health care and breeding related services', while 'livestock/dairy education' receives the tertiary importance. Further, the livestock/veterinary extension system focus on "dairy production" to certain extent, whereas "dairy processing" aspects of extension services were grossly neglected (Subash et al. 2014). Therefore, efficient delivery of dairy extension services has become a matter of great concern and hence, there is a need to understand the role performance of current dairy extension delivery system and to assess their effectiveness in delivering services to farmers. Thus an attempt was made to assess the role performance of various dairy extension service providers in Karnataka State.

Materials and Methods

The present study was conducted in Karnataka State during the period 2019-2020. Based on the physiographic profile of the State, Karnataka was divided into four regions namely, North Zone, South Zone, Central Zone and Coastal & Malnad Zone and from each Zone two Districts (progressive and less progressive districts based on bovine density and milk production density as on 2019) was selected purposively. The selected districts for the study were Belagavi and Gadag from Northern region, Bangalore Rural and Chamarajanagar from Southern region, Davanagere and Chitradurga from Central region Udupi and Uttara Kannada from Coastal and Malnad region representing progressive and less progressive dairy development districts. For the selection of dairy farmers, a multistage stratified random sampling plan was adopted and accordingly from each of the eight districts two Taluks were selected through random sampling method. From each Taluk (cluster of villages) twenty five dairy farmer-respondents were selected randomly thus constituting a total of 400 dairy farmer as respondents for the study. The data were collected by personal interview method using pretested semi-structured interview schedule.

The role performance of various dairy extension service providers existed in the study area, for the purpose of the studying their activities, it was grouped under seven broad categories viz., 'breeding and reproduction services', 'preventive services', 'curative services', 'extension advisory and training services', 'dairy schemes implementation' and 'marketing services'. The primary data pertaining to the different services availed by the respondents in the recent past from various dairy extension service providers was presented and discussed by each service provider. The overall effectiveness the services provided by various service providers was ascertained in terms of 'regularity' in providing services, 'quality', 'timeliness' of the services provided and 'cost effectiveness'. Weighted score for each dairy service delivery was calculated by assigning scores on a three point continuum and then multiplying the percent of observation by the respective score and finally adding the total observation. The weighted mean score was calculated by dividing the sum of total scores for all the indicators of a particular system by the total number of indicators for the particular service and were used for ranking different service providers.

Results and Discussion

Role Performed by Department of Animal Husbandry and Veterinary Services (DAH&VS)

From the table 1, it is evident that breeding and reproduction services was predominantly provided by DAH&VS as perceived by large majority of the dairy farmers who had availed 'Artificial Insemination (A.I)'(87.25%) and 'Pregnancy Diagnosis (P.D) services' (90%) for their milch animals. Similar observation was seen in the findings of Mahalakshmi & Devi (2016) and Karthikeyan et al. (2018) in availing breeding services. In the case of 'preventive services', majority of the dairy farmers availed 'vaccination services' (86%), 'deworming services' (89%) and 76 percent of the respondents opined that the department conducted 'periodic disease surveillance'. Further, majority of them availed the services of 'treatment' (82%) and 'surgery' (72.25%) for their dairy animals from the DAH&VS. With regard to the services pertaining to 'inputs distribution', significant percent of the respondents availed 'veterinary medicines' (87%), 'fodder seeds and stem cuttings' (74%), 'feed supplements'(37%) and other inputs like 'mineral mixtures', 'rubber mats'/'chaff cutter' (29.50%) from the DAH&VS. In the case of provision of 'extension advisory and training services' by the DAH&VS, a significant percentage of the respondents received 'technical guidance on dairy farming' (66.50%), 'organized farmers meeting/demonstrations/field days and visits to exhibitions' (31.25%), 'organized animal health cum infertility camps' (69%), 'organized trainings programmes'(24%), 'farm literature preparation and distribution' (47.25%). Nearly half of the respondents opined DAH&VS involved in implementation of different 'dairy development schemes' (44.50%) and 'provided guidance about credit and insurance facilities' (36.50%). The DAH&VS had played an important role in extending 'breeding and reproduction and health care services' to the farmers in the study area. This might be due to the fact that the mandate of DAH & VS is to provide 'breeding and reproduction and health care services' which were essential and easily accessible to the farmers from the Veterinary hospitals and dispensaries situated in their locality. Similar findings were reported by Umali et al. (1992), Ravikumar & Chander, (2011) and Yadav et.al. (2017) who also observed overwhelming support from public sector in providing breeding and clinical veterinary services.

Role Performed by Dairy Co-operative Societies (DCS) of Karnataka Co-operative Milk Producers Federation Ltd (KMF)

From the results presented in table 1, it is clear that DCS provided access to large majority of the dairy farmer-members to avail the assured 'marketing services'(94%), 'provided technical inputs' viz., veterinary medicines (30%), fodder seeds and stem cuttings (86.50%), feed supplements (89.25%) and other inputs like mineral mixtures, rubber mats/chaff cutter (53.25%). With respect to provision of 'extension advisory and training services' by the DCS, a significant percentage of the respondents received 'technical guidance on dairy farming' (84.50%), 'organized farmers meeting/demonstrations/field days and visits to exhibitions' (45.00%), 'organized animal health cum infertility camps' (87.50%), 'organized trainings programmes'(58.50%), 'farm literature preparation and distribution' (69%). Karthikeyan et al. (2018) and Rathod et al. (2012) also reported that majority of the farmers availed advisory services from dairy co-operatives. A large majority of the member-respondents opined DCS were involved in 'implementation of dairy development schemes' (86%) and provided 'guidance about credit and insurance facilities' (74%). Most of the respondents availed the benefits under Ksheeradhara (milk incentives for milk pourers @ Rs.5/lit of milk poured) programme which is successfully implemented by KMF. Further, a significant per cent (74%) of respondents 'received assistance in availing credit' from nationalized banks/ cooperative banks and insurance facilities where 70 percent of the premium was borne by the State Government and KMF. The DCS had played an important role in extending assured 'marketing services', 'distribution of technical inputs' followed by provision of 'breeding and reproduction and health care' services to the farmers in the study area. This might be due to the fact that Karnataka Co-operative Milk Producers Federation Ltd being a producer company has important role in 'procurement of milk' and 'distribution of subsidized inputs and services' to the member-dairy farmers. Similar findings were observed by Rathod et al. (2012) who were also observed that cooperatives have a significant role in production and marketing activities. A significant majority of the respondents availed 'breeding services viz, 'Artificial Insemination' (79%) and 'Pregnancy Diagnosis' (68%) from Dairy cooperatives. Similar findings were reported by Rathod et al. (2012) that DCS were involved in providing breeding services. Further the dairy cooperatives also played a significant role in assisting DAH&VS in conducting vaccination (51%), deworming (58.50) and periodic disease surveillance (31.75%) programmes and dairy cooperatives also extended the services of 'animal treatment' (80%) and 'surgical procedures' (46.50%) as expressed by majority of respondents. Similar findings were reported by Singodia (2018) that significant percentage of farmers availed preventive (80.35%) and curative services (71.42%) from dairy cooperatives.

Role Performed by NARES (National Agricultural Research and Education System) Institutions

The NARES system comprised of KVK's and Research Institutions of ICAR/SAUs/SUV which played a significant role in providing training and technical guidance to the dairy farmers as well as for the field extension functionaries in the study area. It is evident from table. 1 that NARES institutions played a significant role in provision of 'extension advisory and training services' which included 'technical guidance on dairy farming' (44.50%), 'organized farmers meeting/demonstrations/field days and visits to exhibitions' (62.50%),'organized animal health cum infertility camps' (18.50%), 'organized trainings programmes'(48%), 'farm literature preparation and distribution' (52%). Similar findings were reported by Meena et al. (2019). Further, these institutions were also involved in distribution of inputs viz., 'veterinary medicines' (30%), 'fodder seeds and stem cuttings' (35%), 'feed supplements' (27%) and other inputs like 'mineral mixtures' and 'rubber mats/chaff cutter' (21.50%). The researchers observed that NARES institutions had played a significant however a limited role in reaching out to the dairy farmers through organizing training programmes as well as field extension oriented activities covering the limited geographical area. Further, NARES institutions were primarily involved in capacity development of field extension functionaries through organizing various 'trainers' training programme' on recent advancements in dairying.

Role Performed by NGOs (Non-Governmental Organizations)

The NGOs working in study area were found involved in extending various extension services and provision of inputs to the dairy farmers. Majority of the dairy farmers expressed that NGOs were involved in extending 'breeding and reproduction services' viz., 'Artificial Insemination' (40.50%) and 'Pregnancy Diagnosis' (19.50%) followed by distribution of inputs, 'veterinary medicine' (19%), 'fodder seeds and stem cuttings' (31%), 'feed supplements' (44.25%) and other inputs like 'mineral mixtures', 'rubber mats/chaff cutter' (26.50%). Further, they were also involved in providing 'extension advisory and training services' like, 'technical guidance on dairy farming' (24%), 'organized farmers meeting/demonstrations/field days and visits to exhibitions' (31.50%),'organized animal health cum infertility camps' (13%) and 'organized trainings programmes' (16.50%). The role of NGOs were seen in assisting the dairy farmers in availing the breeding services and also in facilitating them in availing credit services from the organized credit institutions.

Role Performed by Private Consultants

The private veterinarians as consultant/practioners predominantly involved in provided 'breeding and reproduction and 'health care treatment services' to the dairy farmers at their door steps. However, those services were availed by the farmers only during the emergency situations when they were unable to take their cattle to nearest dispensary due to the severity of illness of the animals and other reasons. However the services of private consultants were cost intensive compared to the other governmental agencies and hence their services were used

occasionally. From the table 1 it could be observed that only one fourth of the respondents (A.I 31.50% and P.D 33.50%) availed 'breeding services and reproduction' and 'healthcare services'(Treatment 49% and surgery 28%) from private consultants since adequate breeding and health care services were provided by DAH&VS and DCS in the study area. This finding are in contrary with the findings of Karthikeyan et al. (2018) which revealed that the large majority of respondents

Table 1 Role performance of different service providers in dairy extension delivery system (n= 400)

Sl.No.	Role profile of service providers	DAH&VS	Dairy Co-operatives	NARES	NGO's	Private consultants	Private input dealers/ Private dairies
I Breeding services							
1.	AI services	349(87.25)	316(79.00)	0(0.00)	162(40.50)	126(31.50)	18(4.50)
2.	Pregnancy diagnosis	360(90.00)	272(68.00)	0(0.00)	78(19.50)	134(33.50)	15(3.75)
II Preventive services							
1.	Vaccination programmes	344(86.00)	204(51.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
2.	Deworming	356(89.00)	234(58.50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
3.	Periodic disease surveillance	304(76.00)	127(31.75)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
III Curative services							
1.	Treatment to animals	328(82.00)	320(80.00)	137(34.25)	0(0.00)	196(49.00)	0(0.00)
2.	Performing surgery	289(72.25)	186(46.50)	0(0.00)	0(0.00)	112(28.00)	0(0.00)
IV Input services							
1.	Veterinary medicines	348(87.00)	120(30.00)	120(30.00)	76(19.00)	142(35.50)	272(68.00)
2.	Fodder seeds	296(74.00)	346(86.50)	140(35.00)	124(31.00)	0(0.00)	246(61.50)
3.	Feed supplements	148(37.00)	357(89.25)	108(27.00)	177(44.25)	0(0.00)	296(74.00)
4.	Other inputs	118(29.50)	213(53.25)	86(21.50)	106(26.50)	0(0.00)	184(46.00)
V Extension advisory and training services							
1.	Providing technical guidance to farmers on various aspects of dairying	266(66.50)	338(84.50)	178(44.50)	96(24.00)	240(60.00)	228(57.00)
2.	Organizing farmers meeting/demonstrations /field days and visits to exhibitions	125(31.25)	180(45.00)	250(62.50)	126(31.50)	0(0.00)	0(0.00)
3.	Organizing cattle health cum infertility camps	276(69.00)	350(87.50)	74(18.50)	52(13.00)	0(0.00)	0(0.00)
4.	Organizing training programmes for farmers	96(24.00)	234(58.50)	192(48.00)	66(16.50)	0(0.00)	0(0.00)
5.	Farm literature preparation and distribution	189(47.25)	276(69.00)	208(52.00)	0(0.00)	0(0.00)	0(0.00)
VI Dairy schemes implementation							
1.	Identifying beneficiaries and implementation dairy development schemes	178(44.50)	344(86.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
2.	Providing guidance to avail credit& insurance facilities	146(36.50)	296(74.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
VII Marketing services							
1.	Procurement of milk, storage facility and	0(0.00)	376(94.00)	0(0.00)	0(0.00)	0(0.00)	150(37.50)

Table 2 Overall perceived effectiveness of various services extended by different dairy extension service providers (n= 400)

Sl. No	Dairy Extension Service s provider	Breeding Services		Health care Services		Input Support Services		Extension advisory and training services		Dairy schemes implementation		Marketing services	
		Weighted Mean Score	Rank	Weighted Mean Score	Rank	Weighted Mean Score	Rank	Weighted Mean Score	Rank	Weighted Mean Score	Rank	Weighted Mean Score	Rank
1	DAH&VS	274.26	I	278.67	I	265.25	II	243.95	II	199.16	II	0.00	-
2	Dairy cooperatives	247.91	II	254.70	II	278.33	I	264.35	I	274.53	I	289.73	I
3	NARES	0.00	-	0.00	-	177.18	IV	208.15	III	0.00	-	0.00	-
4	NGOs	112.16	IV	0.00	-	105.88	V	110.12	V	0.00	-	0.00	-
5	Private Consultants	141.96	III	142.43	III	96.04	VI	101.53	VI	0.00	-	0.00	-
6	Private / input dealers Private dairies	104.78	V	0.00	-	214.38	III	184.34	IV	0.00	-	259.30	II

(81.81%) availed health care services offered by the private integrators. Further, they were also found involved in provding veterinary medicines (35.50%) at their door steps and providing technical guidance farmers on various aspects of dairying (60%). The role of private practitioners were largely limited to provision of breeding and reproduction and health related services, supply of veterinary medicines and providing need based technical guidance to the farmers in the study area.

Role Performed by Private Input Dealers/Dairies

Many private input dealers were found operating in the study area and extending timely delivery of input services to the farmers. It could be observed from table. 1 that a significant percent of respondents availed 'veterinary medicines' (68%), 'fodder seeds/ root slips/ stem cuttings' (61.50%),'feed supplements' (74%) and 'other inputs' (46%) from the different input dealers accessible in their locality. In addition to this, private dairies were found playing a significant role in 'procurement of milk', as significant percent of respondents (37.50%) preferred and availed 'marketing services' from private dairies as their payment dues were made comparatively earlier than the dairy cooperative societies. Further, they were also involved in provision of 'extension advisory and training services' (57%) and a small percent of the respondents also availed breeding services.

Overall Perceived Effectiveness of Various Services extended by Different Dairy Extension Service Providers

The overall perceived effectiveness of the dairy service providers were studied based on weighted mean score. From the Table 2 it is evident that with the highest weighted mean score, DAH&VS was ranked first and effective in providing 'health care services' (278.67) and 'breeding and reproduction services' (274.26) and second in 'input support services' (265.26), 'Extension advisory and training services' (243.95) and 'implementation dairy development schemes' (199.16). This may be due to the fact that DAH&VS is mandated in extending health care and breeding and reproduction related services to the dairy farmers on a regular basis with free of cost which makes it more effective. The services of dairy cooperatives was perceived effective and ranked first in providing 'marketing services' (289.73), 'input support services' (278.33), 'dairy schemes implementation' (274.53) and 'extension, advisory and training services' (264.35). Since major role of dairy cooperatives is to provide assured market and remunerative price for the milk produced by the farmer members of the co-operative societies and to provide subsidized input services and extension advisory services the DCS was perceived effective in extending the input and marketing services. Similar findings were also reported by Karthikeyan et al. (2019). The other stakeholders of the dairy extension delivery system, with a limited mandate of extending the services and inputs to the dairy farmers viz., NARES institutions were found more effective in providing 'extension advisory services' (208.15), NGOs in 'breeding services' (112.16),

Private consultants in 'breeding and health care services' (142.43) and private input dealers and dairies in providing 'marketing services' (259.30) and 'input support services' (214.38), respectively.

Conclusions

The DAH&VS of the State played a predominant role in providing breeding and reproduction services and health care services. Whereas, the dairy co-operatives played a vital role in providing assured marketing services, input support services, implementation of dairy schemes and provision of extension advisory and training services to the dairy farmers in the study area. The results of the study clearly indicates that DAH&VS and DCS are two major dairy extension service providers in the study area and were found effective in delivering their services as perceived by the majority of the dairy farmers. However, the services of other service providers viz, NARES institutions, NGOs, Private Consultants and Private input agencies and Dairies were found in extending their limited services to the dairy farmers as per their mandates. However concerted efforts are to be made by all the stakeholders for possible convergence among them in reaching out the dairy farmers and providing emphasis on 'Dairy Extension Education Services' in addition to 'Providing Critical Inputs and other Support Services' to the dairy farmers. Hence, the present study concludes that most of the dairy extension service providers played a significant role in extending different dairy extension services and inputs to the dairy farmers with a different mandate and approaches in reaching out the farmers, most of the time, their efforts were overlapping with other service providers, sporadic in nature and lack of collaboration among these different organizations poses a serious hindrance for the development of dairy sector.

Acknowledgements

The authors thankfully acknowledge ICAR-National Dairy Research Institute for extending all necessary support in conducting this study.

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Indian J Dairy Sci 74(6): 539-545
https://doi.org/10.33785/IJDS.2021.v74i06.011

RESEARCH ARTICLE

Resource use efficiency of milk production across different herd sizes of buffaloes and crossbred cows in Middle Gujarat

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Received: 12 June 2021 / Accepted: 31 October 2021 / Published online: 21 December 2021

Abstract: The most important objective of any production unit is to co-ordinate and utilize the various resources of production in such a manner that they yield the highest net returns. As farmers are shifting from low input-low output to high input-high output and traditional and subsistence to commercial dairy farming, this study assesses the impact of resources being used optimally through herd size categories by the dairy farmers for buffaloes and crossbred cows in the Middle Gujarat region. The resource use efficiency analysis helps the dairy farmers in taking appropriate decisions regarding resource allocation without using additional resources for enhancing their income. The findings show that on an average, the costs of human labour, green fodder and concentrates had significant influence on milk yield for buffalo milk production while costs of human labour, green fodder, concentrates and veterinary and medical charges had significant influence on milk yield for crossbred cow milk production implying that one per cent increase in the use of these inputs can led to increase in the gross returns from milk. Milk production for buffaloes was in decreasing returns to scale while on an overall basis, milk production for crossbred cows was increasing returns to scale. Further, the difference between MVP and its acquisition unit price for human labour in small category, green fodder in overall category and dry fodder in marginal category in buffalo milk production and price for human labour in medium and overall category and green fodder in large category in crossbred cow milk production were found positive and significant indicating that the dairy farmers have an opportunity to increase their profit by using more of these inputs on their farms. Thus, the study concluded that only in crossbred cows, milk production was size neutral.

Keywords: Milk production, Cobb-douglas production function, Resource use efficiency, Returns to scale

Introduction

Over the years, the dairy sector in India has grown substantially and is considered as the most significant economic backbone of rural India. It is also well-known as 'oyster' of the dairy industry worldwide contributing about 28.4 per cent in the agricultural Gross Domestic Product in India and also provides gainful employment all round the year to 16.44 million people (GoI, 2020). Due to the significant negative impact of climate change on agricultural output, the dairy industry has emerged as a reliable source of income and has made significant progress in recent years (Mohapatra et al. (2021)). In order to double the income of the farmers, it is important to focus mainly on the dairy sector especially for the landless labourers, marginal and small categories of farmers and women. To a larger extent, 71% women are involved in most of the work related to animal management which has a significant impact on resource use and its efficiency (GoI, 2012). To be specific, women in India contribute in dairy production ranging from 70.5% in 1993-94 to 76.6% in 2004-05 (GoI, 2017). Dairy sector provides regular income and is an important source of supplementary income to the farmers in the country. India is the global leader in milk production since 1998 achieving an annual output of 198.40 million tonnes during the year 2019-20 (www.indiastat.com). The milk production of the state in the year 2019-20 was 15292000 tonnes (www.indiastat.com) and per capita average milk availability was 595 grams per day during the year 2018-19 (GoG, 2020).

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Among all the species of animals, crossbred cows have been gaining importance in the state milk production. The principal driver of the growth in milk production in the state is largely due to incremental animal numbers. However, a single most significant factor that could augment milk production across any type of species is the yield of the lactating animals (Shah and Dave, 2010). Farmers are shifting from low input-low output to high input-high output and traditional and subsistence to commercial dairy farming. Therefore, to ensure optimal use of various resources by the dairy farmers is of primary concern; helping them taking appropriate decisions regarding resource allocation without using additional resources in enhancing their income. Hence, the present study examines the resource use efficiency in milk production across different herd size categories. The following hypotheses were formulated to examine the resource use efficiency:

H_0: There is no significant difference in the resource use efficiency between different types of milch animals within and across different types of herd size categories

H_a: There is significant difference in the resource use efficiency between different types of milch animals within and across different types of herd size categories

Materials and Methods

Data

This study is based on primary data collected from the dairy farmers during the agricultural year 2018-19 by interviewing the respondents through a pre-tested structured schedule. A multi stage random sampling design procedure was adopted for the study to select the ultimate sampling units. In the first stage, among the nine districts of Middle Gujarat, three districts namely Anand, Kheda and Panchmahal were selected as they constituted highest milk production (Table 1). At the second stage, two talukas were randomly selected from each selected district. In the third stage, four villages were selected purposively ensuring that these villages have buffaloes as well as crossbred cows in the selected talukas. In the fourth stage, from a set of four villages chosen, falling under each taluka, 40 household, 20 each having buffaloes and 20 crossbred cows, were selected in such a way that from each selected category of marginal (1-2), small (3-5), medium (6-10) and large (above 10 milch animals) farmers, 05 households with buffaloes only and 05 households with crossbred cows only were ensured (Lal and Chandel, 2016). The classification of categories was done according to possession of milch animals. Thus, in all, 240 respondents (59 marginal, 60 small, 59 medium and 62 large) spread over 24 villages of three districts comprised as the ultimate sample size.

Methodology

To calculate the resource use efficiency of milk production on the dairy farms, the Cobb-Douglas production function was used taking gross income of milk as dependent variable and other variables *i.e.,* cost of human labour, green fodder, dry fodder, concentrates and cost of veterinary and medical charges as explanatory variables (Kumar and Singh, 2004). The analysis was carried out on per household basis using the SPSS software.

$$Y = a.x_i^{bi}.e^u$$

This helps to capture the ability of the farmer to achieve the maximum realizable output with the given level of inputs under the existing situation and given technology.

The function for variable inputs can be written as:

$$Y = a \cdot x_1^{b1} \cdot x_2^{b2} \cdot x_3^{b3} \cdot x_4^{b4} \cdot x_5^{b5} \cdot e^u \text{ .....(1)}$$

The original equation (1) was converted into natural log form and the parameters were estimated by using the Ordinary Least Squares method.

$$\ln Y = \ln a + b_1 \ln x_1 + b_2 \ln x_2 + b_3 \ln x_3 + b_4 \ln x_4 + b_5 \ln x_5 + U$$

Where,

Y = Gross income from milk per farm per day,

a = Intercept,

X_1 = Cost of human labour (Rs per farm per day)

X_2 = Cost of green fodder (Rs per farm per day)

Table 1 District wise milk production in Middle Gujarat

Name of the Districts	2014-15	2015-16	2016-17	Average	Per cent to Total
Ahmedabad	404.38	425.48	435.83	421.89	14.05
Anand*	523.38	550.88	579.15	551.13	18.35
Dahod	290.34	304.00	298.39	297.57	9.91
Kheda*	622.90	683.58	705.67	670.71	22.34
Panchmahal*	554.93	608.31	644.01	602.41	20.06
Vadodara	451.68	441.16	482.47	458.43	15.27
Total	2847.61	3013.41	3145.52	3002.18	100.00

Source: GoG, 2017

Note: * Considered for study.

X_3 = Cost of dry fodder (Rs per farm per day)

X_4 = Cost of concentrate (Rs per farm per day)

X_5 = Cost of veterinary and medical charges (Rs per farm per day)

$b_1, b_2, \ldots, b_5$ = Regression co-efficients (output elasticity of respective inputs (X_i's))

$\sum_{i=1}^{n} bi$ = Returns to scale, and

e^u = Error term with usual assumptions

u = Random error

A criterion used to measure returns to scale is as follows:

$\Sigma E_p > 1$: increasing returns to scale

$\Sigma E_p = 1$: constant returns to scale

$\Sigma E_p < 1$: decreasing returns to scale.

Marginal value productivity (MVP)

Marginal value product of a particular input was calculated by taking the first order partial derivative of the output (Y) function with respect to corresponding inputs (X_i). The regression co-efficients of inputs obtained were used to calculate marginal value products (MVP) at their geometric mean.

$$MVPxi = bi \frac{\bar{Y}}{\bar{X}}$$

Where,

$\bar{Y}$ = Geometric mean of output (Y),

$\bar{X}$ = Geometric mean of respective inputs (x_i) and

b_i = Regression co-efficient associated with the x_i input.

MVP in relation to marginal factor costs (MFC)

The basic criterion of an efficient resource use is that the MVP of the input just covers the marginal factor cost, which is $MVP_{Xi} = P_{Xi}$. Hence, for evaluating the efficiency of resource use, the ratio of marginal value product for different factors to their respective factor cost was estimated. If the marginal contribution of one unit of input is greater than the price of the input, then the farmers is said to be allocating the resources efficiently and as such there is further scope for allocating more unit of that particular input. If the marginal contribution is negative, then the farmers are said to be using the input excessively so that the fixed resources are no longer responsive to the variable input applied.

The criterion used for determining the optimality of resource use is as follows,

MVP/MFC > 1: under-utilization of resources

MVP/MFC = 1: optimal use of resources

MVP/MFC < 1: over-utilization of resources.

Any deviation of MVP of input i from its unit price may be termed as resource use inefficiency. The higher the difference between MVP of an input and its price, the higher the resource use inefficiency and vice versa. Further, t-statistics was used to test the statistical significance of the difference between the MVP of an input and its unit price. The t-statistics that was used is computed as follows:

$$t = \frac{MVP_{Xi} - P_{Xi}}{SE(MVP_{Xi})}$$

$$SE(MVP_{Xi}) = SE(b_i)\frac{\bar{Y}}{\bar{X}}$$

Where,

MVP_{Xi} = Marginal value productivity of X_i resource,

P_{Xi} = Price per unit of X_i resource,

SE (b_i) = Standard error of regression co-efficient associated with X_i resource and

$\bar{Y}$ and $\bar{X}$ = Geometric means of regression co-efficient associated with X_i resource.

The absolute value of calculated 't' was compared with table value of 't' in case of all inputs at (n-k-1) degrees of freedom where 'n' is the total number of observations and 'k' is the total number of explanatory variables. If calculated 't' was less than table value of 't', then it was concluded that the difference between the marginal value product of a resource and its acquisition unit price were statistically insignificant, indicating the optimal use of this resource and vice-versa.

Results and Discussion

Resource use efficiency

The productivity of resources used for milk production obtained by the production function (intercept, co-efficient of multiple determination and returns to scale) is depicted in the Table 2 for buffaloes and Table 4 for crossbred cows.

The production function for buffalo milk (Table 2) reveals that the co-efficient of multiple determinations (R^2) was 0.92 which

showed that 92.25% variation in the gross return was explained by the model using explanatory variables (X_1 to X_5) in the selected regression model. In case of marginal, small, medium and large size categories, the corresponding values of R^2 were 58.70%, 58.85%, 81.50% and 80.11%, respectively. Further, the results showed that the elasticities were positive as well as negative. It is inferred that among the explanatory variables, cost of green fodder was found positive in all the categories whereas cost of human labour in medium and large categories, cost of dry fodder in large category, cost of concentrates in marginal and small categories and cost of veterinary and medical charges in marginal, small and medium categories were found negative.

The output elasticity of human labour (X_1) was statistically significant at 1% and 5% level of significance in small and overall categories, respectively, which implies the increased usage of labour and thus the gross returns implying that with one per cent increase in labour cost, gross returns will increase by 0.70% and 0.22% in small and overall categories, respectively.

Green fodder appeared to be an important variable influencing buffalo milk production. The elasticity of green fodder (X_2) was statistically significant at 5% and 1% level of significance in large and overall categories, respectively, which implies that one per cent increase in the expenditure on green fodder resulted in an increase of 0.27% and 0.35% in large and overall categories, respectively, in returns from milk production.

On an overall basis, expenditure on dry fodder (X_3) was found to be statistically non-significant. This indicated that there was

Table 2 Production elasticities for different category of farms of buffalo milk

Category of Farm	No. of Farms(n)	Intercept (a)	Production Elasticities (bi)					Σ bi's	R^2
			Human Labour (X_1)	Green Fodder (X_2)	Dry Fodder (X_3)	Concentrates (X_4)	Veterinary & Medical Charges (X_5)		
Marginal	19	1.60	0.23(0.23)	0.26(0.21)	0.38**(0.14)	-0.23(0.23)	-0.02(0.07)	0.62	0.58
Small	15	1.10	0.70***(0.23)	0.11(0.15)	0.11(0.16)	-0.04(0.13)	-0.06(0.06)	0.82	0.58
Medium	15	2.25	-0.14(0.22)	0.15(0.09)	0.13(0.10)	0.26**(0.09)	-0.14*(0.07)	0.26	0.81
Large	21	1.82	-0.09(0.13)	0.27**(0.12)	-0.06(0.08)	0.44***(0.10)	0.06(0.06)	0.62	0.80
Overall	69	0.95	0.22**(0.10)	0.35***(0.06)	0.10(0.07)	0.25***(0.08)	0.02(0.04)	0.95	0.92

Source: Field Survey

Note: Figures in the parentheses indicate standard error of corresponding elasticity.

***Significant at 1 per cent level of significance

**Significant at 5 per cent level of significance

*Significant at 10 per cent level of significance

Table 3 Comparison of MVPs of inputs with their prices for buffalo milk production across herd size categories

Particulars	MVP	Input Price	Difference	Standard Error (MVPxi)	t-Value
Marginal Category					
Cost of Dry Fodder (X_3)	4.10	1.00	3.10	1.51	2.05*
Small Category					
Cost of Human Labour (X_1)	4.29	1.00	3.29	1.41	2.33*
Medium Category					
Cost of Concentrates (X_4)	0.86	1.00	-0.14	0.30	-0.47
Cost of Veterinary and Medical Charges (X_5)	-33.53	1.00	-34.53	16.76	-2.06
Large Category					
Cost of Green Fodder (X_2)	2.11	1.00	1.11	0.94	1.18
Cost of Concentrates (X_4)	1.52	1.00	0.52	0.35	1.51
Overall Category					
Cost of Human Labour (X_1)	1.59	1.00	0.59	0.72	0.82
Cost of Green Fodder (X_2)	3.28	1.00	2.28	0.56	4.06*
Cost of Concentrates (X_4)	0.78	1.00	-0.22	0.25	-0.87

Source: Field Survey

* Significant at 5 per cent level of significance.

no significant impact of feeding of dry fodder to buffaloes on returns from milk production. The reason might be that the data were collected in winter season, which was the flush season for green fodder and farmers were found efficiently utilizing green fodder in the study area. Non-significant impacts of dry fodder on buffaloes were also reported by Ajara and Patel (2008), Thakur and Dhaka (2010) and Sharma et al. (2014).

Among the feed inputs, expenditure on concentrates was observed to be second most important variable significantly influencing returns from milk production. The elasticity of concentrates (X_4) was statistically significant ($P<0.05$) in medium category and statistically highly significant ($P<0.01$) in large and overall categories, implying that one per cent increase in the expenditure on concentrates resulted in an increase of 0.26%, 0.44% and 0.25% in medium, large and overall categories, respectively, in returns from milk production.

On an overall basis, the elasticity of veterinary and medical expenditure (X_5) was found statistically non-significant. Therefore, expenditure on veterinary and medical services had no impact on returns from milk production.

The significant impact of human labour, green fodder and concentrates on returns from buffalo milk production were in conformity with the findings of the earlier research work reported by Ajara and Patel (2008), Parmar et al. (2010) and Kumari and Malhotra (2018).

Returns to scale for buffalo milk production

The value of sum of regression co-efficients (Σ bi's) was observed to be less than one in all the categories of farm indicating that the buffalo milk production is decreasing returns to scale or in other words, sample farmers were observed operating in the second zone of production where area of economic relevance is present within the boundaries of the zone. Zone-II, also called rational zone, depicts somewhere optimum use of inputs representing the range of rational production decisions. Similar results were reported by Parmar et al. (2010).

MVP to MFC for buffalo milk production

A perusal of Table 3 indicates that the difference between MVP and its acquisition unit price for human labour in overall category and green fodder and concentrates in large categories were positive and non-significant indicating that the use of these inputs by the dairy farmers were optimal and being utilized efficiently and there was no need to increase or decrease its use. The positive and non-significant impact of human labour was also reported by Kumari and Malhotra, (2018).

The difference between MVP and its acquisition unit price for human labour in small category, green fodder in overall category and dry fodder in marginal category were positive and significant which indicated that these inputs were being under-utilized implying that there is a scope to further increase the yield of buffalo milk. The positive and significant impact of green fodder was in conformity with the findings of the earlier research work reported by Mehra et al. (2018).

Further, the difference between MVP and its acquisition unit price for concentrates in medium and overall categories were negative and non-significant indicating that concentrates were being over-utilized and there was a need to decrease its use. The negative and non-significant impact of concentrates was also reported by Mehra et al. (2018).

The production function for crossbred cow milk (Table 4) revealed that the co-efficient of multiple determinations (R^2) was 0.92 which showed that 92.94% variation in the gross return was explained by the model using explanatory variables (X_1 to X_5) in the selected regression model. In case of marginal, small, medium and large size categories, the corresponding values of R^2 were 55.85%, 61.93%, 64.15% and 91.49%, respectively. Further, the results showed that the elasticities were positive as well as negative. It

Table 4 Production elasticities for different category of farms of crossbred cow milk

(Rs per farm per day)

Category of Farm	No. of Farms (n)	Intercept (a)	Production Elasticities (bi)					Σ bi's	R^2
			Human Labour (X_1)	Green Fodder (X_2)	Dry Fodder (X_3)	Concen-trates (X_4)	Veterinary & Medical Charges(X_5)		
Marginal	22	0.75	0.49*(0.25)	0.19(0.19)	0.06(0.16)	0.29(0.18)	0.00(0.08)	1.05	0.55
Small	25	1.34	0.32*(0.18)	0.16(0.13)	0.14(0.14)	0.12(0.12)	0.14**(0.06)	0.91	0.61
Medium	23	0.37	0.76***(0.20)	0.06(0.21)	-0.02(0.16)	0.35***(0.13)	0.00(0.04)	1.17	0.64
Large	25	1.26	-0.08(0.18)	0.49***(0.15)	-0.04(0.09)	0.42***(0.15)	0.11**(0.05)	0.90	0.91
Overall	95	0.86	0.37***(0.12)	0.23***(0.08)	0.00(0.08)	0.34***(0.09)	0.04*(0.04)	1.01	0.92

Source: Field Survey

Note: Figures in the parentheses indicate standard error of corresponding elasticity.

*** Significant at 1 per cent level of significance

** Significant at 5 per cent level of significance

* Significant at 10 per cent level of significance

is inferred that among the explanatory variables, cost of human labour was found positive in all the categories except large category whereas cost of dry fodder in medium and large categories were found negative. Therefore, earlier formed hypothesis H_0, that resource use efficiency was same between different types of milch animals within and across different types of herd size categories is rejected.

The output elasticity of human labour (X_1) was statistically significant at 10% level of significance in marginal and small categories and statistically highly significant (P<0.01) in medium and overall categories which implies the increased usage of labour and thus the gross returns implying that with one per cent increase in labour cost, gross returns will increase by 0.49%, 0.32%, 0.76% and 0.37% in marginal, small, medium and overall categories, respectively. Significant impacts of human labour on returns from crossbred cow milk production were in conformity with the findings of Kumari and Malhotra (2018) and Singh and Singh (2018).

The elasticity of green fodder (X_2) was statistically highly significant (P<0.01) for large and overall categories, which implies that one per cent increase in the expenditure on green fodder resulted in an increase of 0.49% and 0.23% in large and overall categories, respectively, in returns from milk production. Thakur and Dhaka (2010) and Sharma et al. (2014) also reported that green fodder was of great importance and had significant impact on crossbred cow milk production.

On an overall basis, expenditure on dry fodder (X_3) was found to be statistically non-significant. This indicated that there was no significant impact of feeding of dry fodder to crossbred cows on returns from milk production. The reason might be that the data were collected in winter season, which was the flush season for green fodder and farmers were found efficiently utilizing green fodder in the study area. Non-significant impacts of dry fodder on crossbred cows were also reported by Ajara and Patel (2008) and Sharma et al. (2014).

The concentrate feed as anticipated, demonstrated its significant positive influence on milk yield in crossbred cows. The high milk producing crossbred cows have an innate high demand for nutrients, which has to be met by concentrate feeds. In realizing higher milk yields from crossbred cows, concentrate feeds have crucial significance. This reveals that for sustaining the milk production from crossbred cows, measures to augment the supply of concentrate feeds are very critical. The elasticity of concentrates (X_4) was statistically highly significant at 1% level of significance in medium, large and overall categories, implying that one per cent increase in the expenditure on concentrates resulted in an increase of 0.35%, 0.42% and 0.34% in medium, large and overall categories, respectively, in returns from milk

Table 5 Comparison of MVPs of inputs with their prices for crossbred cow milk production across herd size categories

Particulars	MVP	Input Price	Difference	Standard Error (MVPxi)	t-Value
Marginal Category					
Cost of Human Labour (X_1)	3.52	1.00	2.52	1.79	1.40
Small Category					
Cost of Human Labour (X_1)	2.43	1.00	1.43	1.37	1.05
Cost of Veterinary and Medical Charges (X_5)	78.43	1.00	77.43	33.61	2.30*
Medium Category					
Cost of Human Labour (X_1)	5.67	1.00	4.67	1.49	3.13**
Cost of Concentrates (X_4)	1.38	1.00	0.38	0.51	0.75
Large Category					
Cost of Green Fodder (X_2)	4.68	1.00	3.68	1.43	2.57**
Cost of Concentrates (X_4)	1.86	1.00	0.86	0.66	1.30
Cost of Veterinary and Medical Charges (X_5)	33.91	1.00	32.91	15.41	2.14*
Overall Category					
Cost of Human Labour (X_1)	2.88	1.00	1.88	0.93	2.01*
Cost of Green Fodder (X_2)	2.12	1.00	1.12	0.74	1.52
Cost of Concentrates (X_4)	1.44	1.00	0.44	0.38	1.15
Cost of Veterinary and Medical Charges (X_5)	16.43	1.00	15.43	16.43	0.94

Source: Field Survey

** Significant at 1 per cent level of significance

* Significant at 5 per cent level of significance

production. Significant impact of concentrates on crossbred cows were reported by Ajara and Patel (2008), Thakur and Dhaka (2010), Sharma et al. (2014), Kumari and Malhotra (2018) and Singh and Singh (2018).

On an overall basis, the elasticity of veterinary and medical expenditure (X_5) was also found to be statistically significant ($P<0.10$). This suggested that on an average, one per cent increase in the expenditure on veterinary and medical services, resulted in an increase of 0.04% returns from milk production in crossbred cows. The significant impact of veterinary and medical expenditure on crossbred cows was also reported by Singh and Singh (2018).

Returns to scale for crossbred cow milk production

In the present study, the value of sum of regression co-efficients (Σ bi's) was observed to be more than one in all the categories of farm except small and large categories, indicating that the crossbred cow milk production is increasing returns to scale or in other words, sample farmers were observed operating in the first zone of production. Zone-I is called irrational zone because the average product increases throughout the zone indicating that the efficiency of all the variable inputs keeps on increasing. So, farmer should not stop in this zone and he must produce up to the level where average product is maximum. Input-use should be continued until zone-II. Hence, it is not reasonable to stop using an input when its efficiency is increasing. If he stops in this region, some of his resource will remain unused or underutilized. In case of crossbred cows on overall basis, 1 per cent increase in the input variables (X_1 to X_5) will result in increase of 1.01 per cent in gross returns. Thus, it is concluded that only in crossbred cows, milk production is size neutral.

MVP to MFC for crossbred cow milk production

A perusal of Table 5 indicates that the difference between MVP and its acquisition unit price for human labour in marginal and small categories, green fodder in overall category, concentrates in medium, large and overall categories and veterinary and medical charges in overall category were positive and non-significant indicating that the use of these inputs by dairy farmers was optimal and being utilized efficiently and there was no need to increase or decrease its use.

Further, the difference between MVP and its acquisition unit price for human labour in medium and overall categories, green fodder in large category and veterinary and medical charges in small and large categories were positive and significant which indicated that these inputs were being under-utilized implying that there is a scope to further increase the yield of crossbred cow milk through increasing the use of human labour, green fodder and veterinary and medical services.

Conclusions

This study analyzed the resource use efficiency of milk production in three districts of Middle Gujarat revealing that there is an ample potentiality of raising milk production on the sample farms through optimum utilization of resources like human labour, fodder and concentrates and with better management practices. Milk production was found to be size neutral only in case of crossbred cows in the region and hence, for increasing farmer's income and eventually livelihood, crossbred cows should be encouraged in the study area. Further, regular studies on resource use efficiency of milk production should be carried out which will help in rational pricing policy.

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Indian J Dairy Sci 74(6): 546-549
https://doi.org/10.33785/IJDS.2021.v74i06.012

SHORT COMMUNICATION

Physico-chemical, antioxidant and sensory properties of stirred yoghurt containing Ber (*Zizyphus mauritiana*) fruit extract

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Received: 12 February 2021 / Accepted: 22 July 2021 / Published online: 21 December 2021

Abstract: In this study polyphenol extract (BPE) of Ber fruit (*Zizyphus mauritiana*) was prepared by vacuum evaporation of ethanol extract of edible portion (pulp and skin) of fruit fortified into stirred yoghurt. A ratio of 0.20 BPE (mg/g) to 1.0 BPE (mg/g) was added for yoghurt preparation. Based on various sensory attributes, stirred yoghurt with BPE 0.8 mg /g per cent was chosen as best among these samples when compared with the control stirred yoghurt. Optimized yoghurt contains 82.36±0.545 % moisture, 6.45±0.08 % protein, 0.98±0.001 % ash, 0.1% fat, 0.026 ppm Mn, 3.85 ppm Zn, 0.9407 ppm Cu and 950 ppm Ca, respectively. Water holding capacity of the fortified yoghurt increased significantly on increasing the BPE concentration from 41.23±0.73g/100g to 46.66 ± 1.13g/100g. Total phenolic content increased significantly on increasing the concentration of BPE 0.8 mg/g. Fortification of BPE resulted in Total Phenolic Content (TPC) of 504.4± 9.3µg GAE/ g from 216.88±9.41µg GAE/ g in control sample. Storage period of 20 days showed non-significant losses in total phenolic content and antioxidant capacity. TPC decreased from 504.4±9.3 µg GAE/g to 438.17±15.58 µg GAE/g and % DPPH inhibition decreased from 74.22 ±2.77% to 67.4 ±1.32% after 20 days storage at 4°C.

Keywords: Stirred yoghurt, Polyphenols, Antioxidant properties, Ber fruit extract, Physico-chemical properties

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Phenolic compounds are very powerful antioxidants due to their hydrogen donating properties and presence of their phenolic hydroxyl groups. Recently these phenolic compounds has gained a significant popularity due to various beneficial properties like antioxidant, anti-mutagen, anti-inflammatory and anti-clotting etc., which is directly related to the risk reduction of cardio vascular diseases and cancer development (Fresco et al. 2010). Fruit and seeds of *Z. jujuba* are reported to have sedative flavonoids compounds such as (4- β-D-glycopyranosyl swetisin) and spinosin. Ten flavonoids were reported from *Zizyphus jujube Lamk* and *Zizyphus spina-chisti* including Querce-tin derivatives; Kaempferol 3-Orobinobioside and Kaempferol 3-O-rutinoside (Pawlowska et al. 2009). Flavonoids have been reported to have beneficial health effects including anti-inflammatory, inhibition of platelet aggregation, inhibition of mast cell histamine release, and antimicrobial activities. Thaipong et al. (2006) reported that phenols in Indian jujube was found to be comparable with fruits already reported to be high in total phenolic content (mg/100 g) e.g. 126–247 in guava, 125–373 in plums. Koley et al. (2011) evaluated 12 commercial cultivars of *Z. mauritiana* for their ascorbic acid (AA), total phenolics (TPH), flavonoids (TF), and total antioxidant activity (AOX). They reported that Indian jujube is a good source of ascorbic acid and total phenolics ranging from 19.54 to 99.49 mg/100 g and 172 to 328.6 mg GAE/100 g, respectively. Total AOX ranged from 7.41 to 13.93 and 8.01 to 15.13 µmol Trolox/g in FRAP and CUPRAC, respectively.

Yoghurt is a fermented dairy product having wide popularity among consumers and can be a suitable product for fortification with various bioactive compounds. (Allegeyer et al. 2010). Addition of fruits extracts in yoghurt will increase its functionality and marketability. Addition of ber polyphenol extracts offer a new way for delivering biologically active phytochemicals to satisfy consumer interest. Therefore, this study reports the effect of addition of ber polyphenols on sensory, physico- chemical and antioxidant properties of stirred yoghurt.

Mature Ber fruits (*Ziziphus mauritiana)* variety Banarasi Karaka were procured from Ber orchard of Banaras Hindu University, Varanasi, Uttar Pradesh, India. Each time the fruits were procured from same orchard to maintain homogeneity in the variety of ber fruits. Spray dried skim milk powder manufactured by Anik Milk

Products Pvt. Ltd. was procured from the local market of Varanasi. Yoghurt starter culture NCDC-144 was purchased from NDRI, Karnal in lyophilized form and were activated before inoculation. Food grade high- methoxy pectin was used for the preparation of stirred yoghurt.

Extraction of polyphenols from ber was done followed the method of Karaaslan et al. (2011) with some modifications. A total phenolic content was determined using a modified version of the Folin- Ciocalteu method (Hinneburg et al. 2006). Folin- Ciocalteu reagent (0.5 mL) was added to appropriately 0.5 mL of diluted sample to form the mixture. After that mixture was kept at room temperature for 5 min and then added 1.5 mL of sodium carbonate (20%) with thorough mixing. The standard calibration (0.02- 0.12 mg/mL) curve was made using gallic acid and results were expressed as µg gallic acid equivalent (µgGAE/g). The scavenging of DPPH radical was carried out according to the method described by Li et al. (2005).

Stirred yoghurt was prepared by following the modified methods of (Sun- Waterhouse, 2013; Zhou, et al. 2011). The stirred yoghurt was formulated with skim milk powder (14.6% w/w), stabilizer (pectin, 0.01% w/w), table sugar (1.0 % w/w), yoghurt starter culture (2% v/v) and Ber Polyphenol Extract (BPE) at varying concentrations. Various samples with different concentration of ber polyphenolic extract (BPE) were coded as below:

Treatment details:

X2B Control) = 0 BPE (mg/g)

XB2 = 0.20 BPE (mg/g)

2XB =0.40 BPE (mg/g)

B2X =0.60 BPE (mg/g)

2BX =0.80 BPE (mg/g)

BX2 =1.0 BPE (mg/g)

Sensory evaluation of optimized yoghurt was done using 9.0 Point-Hedonic Scale. Yoghurt samples were analyzed for color & appearance, flavour, consistency & mouth feel and overall acceptability after overnight storage at 4-5 °C. Different physicochemical parameters such as % fat, % Acidity, % Protein, % ash content, % total solids, % moisture, viscosity, and pH were determined as per the methods described by Rangana (2001). Minerals were determined using Atomic Absorption Spectroscopy (Thermo Fisher Scientist-IN). Total phenolic content of stirred yoghurt was analysed by Folin-Ciocalteau method Xia et al. (2010) with some modifications. Antioxidant activity was accounted in terms of percent of DPPH inhibition method Nishino et al. (2000). Textural parameters of BPE fortified yoghurt were analyzed using Texture Analyzer (TA.XT *plus* texture profile analyzer, Stable Micro System Ltd, Model TA-XT plus, UK) with probe back extrusion Rig 35 mm Disc. Water holding capacity of yoghurt was determined by method of Isanga et al. (2009). The yoghurt susceptibility to syneresis (STS) was measured by placing a 100 ml of yoghurt sample on a filter paper placed on a funnel. After 6 h of drainage, the volume of the whey collected in a beaker was measured and used as an index of syneresis.

The data of the analyses were pooled and averaged and the mean and standard deviation were calculated using MS Excel software and an analysis of variance (one-way ANOVA) and differences at ($p<0.05$) were considered significant

Concentrated ethanolic extract of ber homogenate (pulp including skin) was prepared by vacuum evaporation. The total phenolic content of concentrated extract prepared from edible portion (skin and peel) of variety Banarasi Kadaka was found to be 325.20± 25.43 µg GAE/ ml, % DPPH, 71.09 ± 1.89, ascorbic acid, 86.38 ± 2.69 (mg/100g), respectively. Minimum changes in physico-chemical properties of extract were observed during recommended storage at -20°C. Similarly, Koley et al. (2011) reported the total phenolic content of Indian Jujube (*Zizyphus mauritiana* Lamk.) ranging between 172 to 328.6 mg GAE/g among various cultivars. Thaipong et al. (2006) also reported that Ber is found to be comparable with fruits already reported to be high in TPC like

Table 1 Physico–chemical, antioxidant and texture analysis BPE fortified stirred yoghurt

Composition	Optimized (BPE@0.8%) fortified yoghurt
pH	4.88±0.03
Acidity (% lactic acid)	0.86±0.02
Total solids	15.33±0.25
Moisture (%)	82.36±0.54
Protein (%)	6.45±0.08
Ash (%)	0.98±0.001
Fat (%)	0.10±0.08
Mn (ppm)	0.026±0.01
Zn (ppm)	3.85±0.03
Cu (ppm)	0.94±0.01
Ca (ppm)	950±0.02
Water holding capacity(ml/100ml)	46.42 ± 1.03
Susceptibility to syneresis (g/100 g)	42.91±1.84
Total phenolic content (µg GAE/g)	504.4±9.30
DPPH inhibition (%)	74.22±2.77
Water holding capacity(ml/100ml)	46.42 ± 1.03
Texture profile of BPE fortified	stirred yoghurt
Firmness (g)	44.07±2.83
Consistency (g sec)	893.493±16.0
Cohesiveness (g)	46.56±6.95
Index of viscosity (g sec)	105.39±7.62

Data represents Mean ± standard deviation of 3 replications

Table 2 Effect of BPE concentration on various properties of stirred yoghurt

Level of incorporation BPE (mg/g)	pH	Acidity (as % lactic acid)	Total solids (%)	WHC (%)	Susceptibility to synersis (ml/100ml)	DPPH inhibition (%)	TPC (µg GAE/g)
0	4.83±0.04	1.09±0.03	14.83±0.50	41.23 ±0.73^{a}	45.15±0.59^{a}	23.26±6.00^{e}	216.88±9.41
0.2	4.87±0.07	0.97±0.02	15.00±0.36	41.09 ± 0.55^{a}	42.57±1.11^{b}	60.33±5.07^{d}	299.95±20.05
0.4	4.85±0.01	0.92±0.02	14.97±0.21	45.77 ± 1.01^{a}	44.13±0.45^{ab}	66.59±1.56cd	346.53± 19.42
0.6	4.88±0.02	0.87±0.02	15.07±0.31	46.34 ± 0.67^{b}	43.71±0.50^{ab}	70.49±3.04bc	473.16± 15.92
0.8	4.88±0.03	0.86±0.02	15.33±0.25	46.42 ± 1.03^{b}	42.91±1.84^{b}	74.22±2.77ab	504.4±9.30
1.0	4.86±0.09	0.81±0.02	15.37±0.25	46.66 ± 1.13^{b}	45.177±1.21^{a}	80.35±1.18a	525.87± 7.86

Mean ± S.D; means with different superscripts in a column differ significantly ($p< 0.05$) (n=3)

guava and plums. Koley et al. (2016) reported that Ber fruit is a good source of ascorbic acid and total penolics ranged from 19.54 to 99.49 mg/100g and 172 to 328.6 mg GAE/100g, respectively. The ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) of Ber fruit were ranged from 7.41 to 13.93 and 8.01 to 15.13 µmol Trolox/g, respectively.

Variant 2BX was found to have highest sensory preference, while 2XB has the lowest values. The value for X2B (control) sample was almost same for overall acceptability as 2BX. The change in the color and appearance was found to be significant ($p<0.05$) over various added concentrations of BPE in yoghurts. The flavor of stirred yoghurt was found to be changed non-significantly ($p>0.05$). Sample 2BX had highest sensory score while XB2 had the lowest value. At higher concentrations, the flavor may be enhanced and appreciated up to a certain concentration. The yoghurt variant with highest overall acceptability containing 0.80 BPE (mg/g) other than control (0% extract) was chosen as optimized yoghurt for further study.

Table 1 shows the proximate analysis of optimized yoghurt. The mineral composition in terms of Mn, Zn, Cu and Ca for BPE fortified yoghurt was found to be comparable to literature values for yoghurts (Segarra et al. 2000). Optimized yoghurt contained total phenolic content 504.4±9.30 (µg GAE/g), DPPH inhibition 74.22±2.77 %, respectively. Based on sensory scores for consistency, the control and optimized yoghurt were selected for the textural analysis. The peak force during the first compression cycle is defined as firmness. The firmness of control yoghurt was found to be significantly higher ($p< 0.05$) than extract fortified yoghurts and ranged between 39.46 and 94.73g. Optimized and control yoghurt were analyzed for consistency. The firmness of the yoghurt was found to be inversely proportional to the concentration of extract. This can be attributed to the low fat amounts and the natural properties of the extract. Consistency of control sample i.e.1736±46.92 g sec. was significantly higher than extract fortified samples, 893.493±16.0 g sec ($p< 0.05$). Cohesiveness of control sample (66.11±3.27 g) was significantly higher ($p< 0.05$) than fortified yoghurt samples. Index of viscosity was found to be maximum for control sample 180.15 g. sec. Index of viscosity was 107.29 g. sec with addition of 0.4 mg/g BPE which shows significant decrease i.e. 105.39 g. sec with addition of 1.0 mg/g BPE in yoghurt.

It was reported that the higher the levels of solids in the yoghurt mix, the greater the viscosity/consistency of the end product (Tamime & Robinson, 1999). In this study, the level of total soluble solids of yoghurt mix was maintained 14.83±0.50, before the addition of the BPE. The concentration of extract up to 1.0 mg/g does not show a significant change in pH ($p>0.05$) but a slight decline in acidity was noticed on increasing the concentration of extract, which can be attributed to the natural acidity of the fruit. This change was found to be significant ($p<0.05$). Similar observations were reported by (Yadav et al. 2016). They observed that pH and acidity are found to be inversely effected on addition of grape peel extract in the stirred yoghurt. Total solids changes with increase in BPE concentration in stirred yoghurt but this change was not significant ($p>0.05$). Generally, the water holding capacity (WHC) of control as well as fortified yoghurt were low and addition of pectin (0.01%) may have improved the hydration behavior. A ($p<0.05$) significant increase in water holding capacity was observed on increasing the extract concentration in stirred yoghurt. The trend of increase was maximum at the lower level of extract addition and got stable at higher level. The effect of addition of BPE showed significant ($p<0.05$) effect on syneresis.

In present study, polyphenol extract from Ber fruit's edible part (pulp and skin) was added to the stirred yoghurt before fermentation. Sun-Waterhouse (2013) and Zhou, et al. (2011) explored two approaches of adding polyphenols into yoghurt: via the pre-fermentation approach or via the post fermentation approach. The addition of BPE to the yoghurt increased the polyphenol content significantly ($p<0.05$).The increase in the concentration of BPE positively correlates with the TPC content ($R^2 = 0.9523$). Table 2 shows the results for polyphenolic content in various samples of stirred yoghurt having different level of BPE extract. Stirred yoghurt with 1.0 mg/g BPE content was found to have maximum TPC of 525.87± 7.86 µg GAE/g. Addition of BPE at the rate of 1.0 mg/g had the maximum value (80.35±1.18%) for % DPPH inhibition. The radical scavenging capacity had increased significantly ($p< 0.05$) on increasing the percentage of extract in

the yoghurt, but these factors were not perfectly correlated (R2=0.7522).

Conclusions

From this study, it is concluded that polyphenol rich extract can be obtained from Ber (*Zizyphus mauritiana*) and can be successfully incorporated in stirred yoghurts for polyphenolic fortification. Polyphenol fortified stirred yoghurt, thus can contribute to meet the polyphenols requirement of the body.

Funding

Sincere thanks are extended to Institutions of Eminence (IoE) scheme, Banaras Hindu University, Varanasi (U.P) India, for financial support under Incentive to Senior Faculties under IoE.

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https://doi.org/10.33785/IJDS.2021.v74i06.013

SHORT COMMUNICATION

Effect of graded levels of dietary crude protein on nutrient utilization and enteric methane emissions in growing Murrah buffalo calves

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Received: 27 March 2021 / Accepted: 16 September 2021 / Published online: 21 December 2021

Abstract: The present study was done to investigate the effect of graded levels of dietary crude protein on enteric methane (CH_4) production from Murrah buffalo calves. Fifteen Murrah buffalo male calves were divided into 5 groups (Av. BW=153.05) and were fed total mixed rations containing wheat straw, maize fodder and concentrate mixture in different proportions so that the dietary crude protein level was 5, 7.5, 10, 12.5 and 15% in groups T_1,T_2,T_3,T_4 and T_5, respectively. The trial lasted for 30 days. Dry matter intake increased from 2.49 to 4.40 kg/d. Dry matter digestibility increased ($P<0.05$) from 48.64 to 61.97%. CH_4 emissions decreased ($P<0.05$) from 34.48 to 12.73 g/kg DMI with increasing protein level in the ration. Hence, CH_4 emissions were lower (12.73-12.83 g/Kg DMI) in animals fed rations containing 12.5 to 15% CP.

Keywords: Buffalo calves, Enteric CH_4 emissions, Nutrient utilization

Concern of world environmental authorities towards increasing level of green house gases (GHGs) in the atmosphere is due to their global warming impacts. CH_4 is the second most important greenhouse gas after carbon dioxide due to its 25 times higher global warming potential (Prathap et al. 2021). Ruminant livestock constitute the most important source of anthropogenic emissions of CH_4 (Broucek, 2014) and enteric fermentation and manure management (along with nitrous oxide) are the two responsible forms (Nampoothiri et al. 2018).

Due to prevailing feeding practices in the country where poor quality roughages forms major constituents of animal diet, production of acetate is more which contributes to higher CH_4 production and poor productivity of animals (Garg et al. 2012). Imbalanced feeding resulted in low milk production, poor growth and reproduction, shorter lactation length, longer calving intervals and excessive amounts of pollutants released into the environment (Gupta et al. 2019). Though biotechnological and management methods are quite tedious, manipulating methanogenic causes through feed interventions are more researched and adoptive ways (Prathap et al. 2021). Supplementation of concentrate or increasing the dietary crude protein in the ration is being practiced over the years to reduce CH_4 emissions (Muñoz et al. 2015). Thus, the present study was aimed to investigate the effect of using different crude protein in the ration on nutrient utilization and CH_4 production in Murrah buffalo calves.

Fifteen male Murrah buffalo calves (153.05 kg BW, 6-8 mon.) at Livestock Research Centre (LRC) of ICAR-National Dairy Research Institute (NDRI), Karnal, India, were distributed randomly in five groups of three each based on body weight and age. The animals were fed on graded levels of protein in the ration i.e. total mixed rations containing wheat straw, maize green and concentrate mixture in different proportions so that the dietary CP level was 5, 7.5, 10, 12.5 and 15% of DMI in groups T_1 (R:C=100:0), T_2 (R:C=80:20), T_3 (R:C=70:30), T_4 (R:C=65:35) and T_5 (R:C=55:45), respectively. The feeding trial lasted for 30 days, followed by a 7 day metabolic trial. Dry matter intake was recorded daily for each animal. The samples of feed, residue, feces and urine were analyzed for proximate principles (AOAC 2005) and NDF (Van Soest et al. 1991). Non-fibrous carbohydrates (NFC), total digestible nutrients (TDN), digestible energy (DE) and metabolizable energy (ME) values were calculated from feed composition using equations (NRC, 2001).

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Enteric CH_4 emission was measured for a total of 5 days (*i.e.* minimum of 5 samples per animal) using SF_6 tracer technique (Johnson et al. 1994). Collected samples (gases eructed from the mouth and nostrils) were analyzed for CH_4 and SF_6 gas using chromatograph (Nucon-5700, New Delhi) fitted with flame ionization detector (FID at 100°C) and electron capture detector (ECD at 250°C), respectively. Nitrogen was used as the carrier gas at a pressure of 1 kg/cm^2. The CH_4 emission rate was calculated as:

$$CH_4 (g/day) = (S_{CH4}-B_{CH4})/(S_{SF6}-B_{SF6}) \times (M_{CH4}/M_{SF6}) \times Q_{SF6} \times 1000$$

Where, S_{CH4} and B_{CH4} are CH_4 concentrations in sample and background's canisters; S_{SF6} and B_{SF6} represents the concentrations of SF_6 in sample and background's canisters (ppt), respectively and Q_{SF6} represents the release rate of SF_6 (mg/d).

Statistical analysis was done using completely randomized design, one-way classification as per the procedure given by Snedecor and Cochran (1994). Significant differences among different treatments were identified using Duncan's Multiple Range Test and a $p<0.05$ was considered to be statistically significant. All the statistical analyses were done using SPSS version 16 (2010).

Concentrate mixture, maize green and wheat straw were fed to the five groups in the form of TMR in such a fashion so as to provide a total dietary CP level of 5, 7.5, 10, 12.5 and 15% in groups T_1, T_2, T_3, T_4 and T_5, respectively. Chemical composition and the energy content of the feeds have been presented in Table 1 and were in agreement with the earlier reports (Dixit et al. 2015; Budhani et al. 2016).

Nutrient intake and digestibility figures have been shown in Table 2. The DM intake was the lowest ($P<0.05$) in group T_1 (2.49 kg/d) and the differences among other groups were not significant, however, CP intake increased ($P<0.05$) with increasing levels of protein in the diet. Feed intake is partially dictated by physical capacity of the rumen, especially, in higher forage diets which might have reduced DMI (Nampoothiri et al. 2018).

Table 1 Chemical composition of experimental feeds (% DM basis)

Attribute	Concentrate mixture	Maize fodder	Wheat straw
Chemical composition (%)			
DM	91.36±0.51	24.14±0.78	93.05±0.04
OM	90.69±0.41	88.67±0.12	86.41±0.15
CP	20.86±0.04	8.14±0.54	3.02±0.47
EE	5.02±0.10	1.45±0.80	1.02±0.15
TA	9.31±0.63	11.33±0.05	12.59±0.85
NFC	37.53±0.18	21.44±0.10	10.72±0.01
NDF	25.83±0.16	56.63±0.24	70.66±0.81
ADF	13.67±0.77	28.41±0.35	42.61±0.84
Energy content			
TDN (%)	74.59±1.96	52.10±2.03	41.70±0.88
DE (MJ/Kg DM)	14.32±0.89	9.69±0.54	7.66±0.12
ME (MJ/Kg DM)	12.62±0.30	7.87±0.26	5.82±0.17

DM=Dry Matter; TA=Total Ash; OM=Organic Matter; CP=Crude Protein; EE=Ether Extract; NDF=Neutral Detergent Fiber; ADF=Acid Detergent Fiber; NFC=Non-Fibrous Carbohydrates; TDN=Total Digestible Nutrients; DE=Digestible Energy; ME=Metabolizable Energy

Table 2 Nutrient intake and digestibility in buffaloes given different levels of protein in diet

Particular	T_1	T_2	T_3	T_4	T_5
BW (kg)	151.22±2.67	152.23±4.01	156.21±3.78	155.51±4.08	150.08±3.62
Nutrient intake					
DMI (kg)	2.49^{a}±0.28	3.38^{ab}±0.29	4.04^{b}±0.24	4.26^{b}±0.29	4.40^{b}±0.59
CP intake (g/day)	100.69^{a} ±3.54	257.28^{b} ±36.85	400.28^{c}±51.40	519.89^{d}±64.44	587.49^{d}±76.14
Digestibility of nutrients (%)					
DM	48.64^{a}±0.77	50.45^{a}±1.36	55.07^{ab}±0.56	60.67^{c}±1.57	61.97^{c}±0.91
OM	50.90^{a}±0.12	53.17^{b}±0.94	57.01^{b}±1.78	63.56^{c}±0.49	65.61^{c}±0.17
CP	45.45^{a}±3.01	70.32^{b}±1.24	75.47^{bc}±2.67	80.18^{c}±1.45	81.94^{c}±1.08
NDF	50.23±2.98	53.74±2.69	53.19±4.78	56.77±3.29	50.62±3.94
ADF	43.69±1.99	46.95±5.01	43.78±1.33	45.45±1.45	43.86±3.28

^{abc}Means bearing different superscripts in the same row differ significantly ($P<0.05$)

Table 3 Enteric CH_4 emissions in calves as affected by crude protein content in the rations

Particular	T_1	T_2	T_3	T_4	T_5
CH_4 (g/d)	84.07^{a}±1.95	66.93^{b}±1.17	61.80^{c}±0.71	54.07^{d}±1.23	54.40^{d}±2.06
CH_4(g/kg DMI)	34.48^{a}±3.45	20.08^{b}±1.52	15.39^{bc}±0.70	12.83^{c}±0.99	12.73^{c}±1.34
CH_4 loss					
% of GE intake	11.75^{a}±1.23	6.66^{b}±0.74	5.04^{bc}±0.44	4.32^{c}±0.82	3.95^{c}±0.37
% of DE intake	23.00^{a}±3.39	11.58^{b}±1.36	8.19^{c}±1.57	6.61^{d}±0.47	5.58^{d}±0.67
% of ME intake	29.75^{a}±3.97	14.34^{b}±1.59	9.92^{c}±1.88	7.87^{d}±0.94	6.94^{d}±0.36

^{abcd}Means bearing different superscripts in the same row differ significantly (P<0.05)

Enteric CH_4 emissions data have been presented in Table 3. On an average, CH_4 emission in groups T_3 to T_5 (12.73-15.39 g/kg DMI) found in agreement in case of growing Murrah calves i.e. 9.47-13.6 g/kg DMI (Nampoothiri et al. 2018), 12.2-13.3 g/kg DMI (Gupta et al. 2019) and 11.43-16.17 g/kg DMI (Budhani et al. 2016); however, CH_4 production in groups T_1 and T_2 were not comparable, might be due to low crude protein feed structure than the average feeding patterns of the rations. CH_4 loss represented similar loss of GE intake in the previous work (Appuhamy et al. 2016) resulting in reduced feed efficiency in the animals.

Thakur et al. (2021) found that increasing concentrate portion from 70-20% in the ration reduced the enteric CH_4 emission by 34.18% in crossbred kids. However, enteric CH_4 production was not affected by increasing CP content (14.1-18.1%) in the concentrate (Hynes et al. 2016). By selecting higher feed efficient animals, CH_4 production in the fermentation process of rumen can be reduced by decreasing CP content (130-175 g/kg DM) of the ration due to less urinary nitrogen losses (Kidane et al. 2018). Similar results have been reported (Niu et al. 2016) but with a higher level of CP (18.5%) in the diet.

Shifting the ration from concentrates to fibrous feed, higher acetate and butyrate levels, being the precursor for CH_4 production in the rumen levels caused higher CH_4 emissions (Dijkstra et al. 2011). Decreased CH_4 production by increasing CP content did not affect CH_4 emission in absolute terms i.e. L/day but there was reduction in CH_4 emission as a proportion of DMI, milk yield and GE intake (Sherasia and Garg 2016) as availability of grains causes formation of more propionate which does not provide H_2 for methanogenesis (Niu et al. 2016). However, accounting CH_4 emission per kg milk produced make sense only in the higher milk production animals (Muñoz et al. 2015) which negotiated the CH_4 production with higher milk yield. Higher grain content of concentrate led to increase in starch level in rumen, thereby, reducing ruminal pH and activity of protozoa and methanogens (Uddin et al. 2020).

Conclusions

Increasing proportion of dietary CP in the ration increased dry matter intake, nutrient intake and nutrient digestibility along with reduction in CH_4 emissions in growing Murrah buffaloes. Therefore, 12.5-15% CP in the ration of growing Murrah buffaloes may be recommended based on the results of present study.

Acknowledgements

The authors thank Director, ICAR-National Dairy Research Institute, Karnal, Haryana, India for the financial support.

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Indian J Dairy Sci 74(6): 554-555
https://doi.org/10.33785/IJDS.2021.v74i06.014

SHORT COMMUNICATION

Exon 10 prolactin receptor gene polymorphism in Surti and Jaffarabadi buffaloes

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Received: 19 July 2021 / Accepted: 30 August 2021 / Published online: 21 December 2021

Abstract: The present study was undertaken with the objectives to amplify Prolactin Receptor (PRLR) gene exon 10 region and to study PRLR gene polymorphism using PCR-RFLP in Surti and Jaffarabadi buffaloes. Blood samples from 50 Surti and Jaffarabadi buffaloes each were collected followed by DNA was extracted by phenol: chloroform method. Oligo primers specific to bovine PRLR loci were custom synthesized at Eurofins and utilized in the present experiment. Exon 10 region of PRLR gene resulted in 168 bp product. Restriction digestion of 168 bp fragment of PRLR gene using SmlI, revealed PCR products with a single band of GG genotype. The frequency of genotype GG and gene G were observed 1.0 each for both Surti and Jaffarabadi buffalo respectively. Both the population of buffaloes were found to be in genetic equilibrium for PRLR gene of exon 10 locus indicating that monomorphism at these loci may be a species characteristic of buffalo.

Keywords: Buffalo, Prolactin receptor, Polymorphism, PCR-RFLP

India with rich domestic animal genetic resources and which are associated with the social, cultural and traditional values of the region to which they belong and serve as vital source for food, draught power, manure and provide much needed self-employment to small and marginal farmers (Anonymous 2020). In India buffaloes are preferred over cattle as a dairy animal and are called "The black gold of India" because of high milk fat content which fetches higher market price.

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Development in DNA technologies have made it possible to uncover a large number of genetic polymorphisms at the DNA sequence level and to use them as markers for evaluation of the genetic basis for observed phenotypic variability which makes a vital part in animal breeding (Athe et al. 2018). Many candidate genes with putative effects on economically important traits have been investigated in many cattle and buffalo breeds, some were reported to affect milk production traits e.g. PRLR, PRL, LEPR, DGAT1 etc. Prolactin (PRL) and Prolactin Receptor (PRLR) are peptide hormones secreted from the anterior pituitary gland and play an important regulatory function in mammary gland development, maintenance of milk secretion and reproduction.

Several polymorphic sites have been detected in different buffalo breeds and reported Single Nucleotide Polymorphisms (SNPs) in PRLR gene including Murrah and Mehsana (Javed et al. 2011a; Javed et al. 2011b), Murrah and Nili-Ravi and crossbred buffaloes (Abakar et al. 2018), Italian Mediterranean River buffaloes (Cosenza et al. 2018) and Egyptian Buffaloes (El-Magd et al. 2021).

The Surti and Jaffarabadi are two divergent buffalo breeds of Gujarat having star performance status as the premier dairy animal of the India. However, there is no report available in Surti and Jaffarabadi buffalo breeds of PRLR gene polymorphism and its effects on milk production traits. The present study was undertaken with the objectives to amplify PRLR gene exon 10 region and to study PRLR gene polymorphism using Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP) in Surti and Jaffarabadi buffaloes.

Surti buffaloes, maintained at Livestock Research Station under Navsari Agricultural University, Navsari and Jaffarabadi buffaloes, maintained at Patel Dairy Farm, Navsari, were used in present research work. About 5ml of the blood was collected from each animal from the jugular vein and genomic DNA was isolated from blood samples using standard phenol-chloroform extraction method by John et al. (1991). The concentration and purity of genomic DNA was determined spectrophotometrically at OD_{260} and OD_{280}.

Oligo primers specific to bovine PRLR loci utilized in the present study (F- 5'AGATGGAGTGCTGGCTCTGT3' and R-

Table 1 Gene and genotypic frequencies of PRLR gene

Breed (n=50)	Observed no. of genotype			Genotypic Frequency			Gene Frequency		Expected no. of genotype		
	GG	GT	TT	GG	GT	TT	G	T	GG	GT	TT
Surti	50	00	00	1.00	0.00	0.00	1.0	0.0	50	00	00
Jaffarabadi	50	00	00	1.00	0.00	0.00	1.0	0.0	50	00	00

5'GCCTTCTTGGCTGGTTCTTC3') as per Parihar et al. (2017) were custom synthesized by Eurofins Genomics. PCR reaction was carried out in total volume of 25 µl that included 12.5 µl of 2X master mix (by Takara Ltd.), 3 µl of genomic DNA (90 ng), 1 µl of each forward and reverse primer (10 pmole) and 7.5 µl of nuclease free water with annealing temperature of 60^0C.

Restriction fragment digestion of amplified products (168 bp) were carried with SmlI (Thermo Scientific Biolab) out in a total reaction volume of 21 µl at incubation of 30^0C for 16 hrs followed by inactivation of 65^0C for 20 min. Digested products were checked on 2.0% agarose gel in 0.5 X TBE buffer for 60 to 90 minutes at 5 V/cm. The band size were judged by comparing with 50 bp DNA ladder and recorded. Genotyping of PRLR loci were carried out according to the band pattern of respective genotypes.

The amplified fragments of the PRLR gene exon 10 region revealed about 168 bp PCR product in both Surti and Jaffrabadi buffaloe breeds. As per Deepika et al. (2014), SmlI restriction analysis of the PCR product yields three different genotypes viz., GG genotype (168 bp), GT genotype (168, 123 and 45 bps) and TT genotype (123 and 45 bps).

In present study on restriction digestion of exon 10 region of PRLR gene using SmlI revealed RFLP pattern with only one genotype (GG) and one allele (G) for the locus in both Surti and Jaffarabadi buffaloes as presented in table 1. Screened buffalo population used in present study was found monomorphic in nature.

In contrast to the present study, Javed et al. (2011a; 2011b) reported six buffalo- specific SNPs in PRLR gene in B. bubalis . Abakar et al. (2018) detected a SNP at 9637 (T/C) in exons 10 of PRLR gene in Murrah, Nili-Ravi, and crossbred buffaloes. Cosenza et al. (2018) sequenced and detected the SNP in exon 10 in 308 Italian Mediterranean river buffaloes. El-Magd et al. (2021) detected two SNPs in exon 10 of PRLR gene in Egyptian Buffaloes.

Associations between PRLR variants and milk production traits have been reported by various authors. Abakar et al. (2018) reported association in PRLR exon 10 in Murrah buffaloes with milk yield, with a significant difference between the TT and CC genotypes. Xinfeng et al. (2020) reported that individuals with genotype DD showed better phenotypic traits than individuals with other genotypes at the two loci of PRLR gene in Shaanbei white cashmere goats. El-Magd et al. (2021) reported Egyptian buffaloes with GT haplotypes showed significantly higher milk yield, fat% and protein%, mRNA and protein levels PRLR in milk somatic cells than AC, AT and GC haplotypes.

Conclusion

PRLR 168 bp region (exon 10) amplified with bovine specific primers showed monomorphic restriction pattern on digestion with SmlI enzyme in Surti and Jaffarabadi buffaloes. Genotypic (GG) and gene (G) frequencies were recorded as 1.0 each in Surti and Jaffarabadi buffaloes respectively. It could be inferred that further study is required to validate PRLR gene to be used as molecular marker in selection to improve the milk production performance in Buffaloes.

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Authors are responsible for including the names of persons who have made substantial contributions to the study orInstitutions which have given financial assistance to the work.

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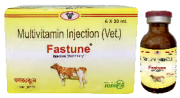

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ISSN 0019-5146 (Print)
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