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INVITED REVIEW

Staphylococcus aureus: significance, control and rapid detection across milk chain

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Abstract: *Staphylococcus aureus* is a major pathogen of public health concern with dominant role in food poisoning outbreaks, nosocomial and community-acquired infections, and also in bovine mastitis. Milk being a rich nutritious source for growth and proliferation of pathogenic species is prone to bacterial contamination, which can lead to spoilage and food poisoning. Toxin production, heat resistance, biofilm formation, antibiotic and lysozyme resistance are among the characteristics that contribute to *S. aureus* pathogenicity. *S. aureus* and its enterotoxins in food remains a daunting challenge, despite global efforts towards its mitigation. There is a need for rapid and cost-effective on-site detection of *S. aureus* in milk to prevent its transmission. The article outlines the significance of *S. aureus* in milk chain, its ability to adapt to environmental stresses, possible mitigation and rapid detection strategies; that may help to curtail its presence in milk chain and linked food poisoning episodes; besides ensuring food quality and consumer safety.

Keywords: Dairy; Pathogen detection; Food-poisoning; Food-borne illness; Food safety; Mastitis; Milk; *Staphylococcus aureus*; Antimicrobial resistance

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Abbreviations: AuNPs, gold nanoparticles; Aw, Water activity; CFU, colony forming unit; D value, decimal reduction value; dsDNA, double stranded DNA; FDA, Food and Drug Administration; LAMP, Loop Mediated Isothermal Amplification; MRSA, Methicillin Resistant *Staphylococcus aureus*; *S. aureus*, *Staphylococcus aureus*; WHO, World Health Organization

Introduction

With globalization and growing awareness among consumers for variety and quality in foods, there is surge in food trade across borders resulting in need for processed and packaged food with extended shelf life. With this expanding food supply chain, the potential for spread of food borne pathogens is high and demands attention. Unsafe food can lead to episodes of food poisoning, malnutrition, economic losses due to rejections, withdrawals etc. and consumer dis-satisfaction. Food safety remains one of the most important global health issues, and food-borne diseases caused by microbes are a widespread public health concern. *S. aureus* is amongst one of the leading cause for food poisoning and other infections and is a major focus for public health programs worldwide (Xihong et al. 2013). *S. aureus* mediated food poisoning episodes are routinely witnessed globally. Recently, Fusco et al. (2020) documented past 20 years data for the food poisoning outbreaks linked to consumption of milk and milk products contaminated with *Staphylococcus* spp. Although *S. aureus* has been associated with food poisoning in various foods, but Milk has been shown to be at risk for *S. aureus* contamination (Gill et al. 1994a; Xie et al. 2021). This is because milk acts as rich media for growth and proliferation of pathogenic species, which can lead to spoilage and food poisoning (Girma et al. 2014). Intoxication by staphylococcal enterotoxins is one of the most common causes of food poisoning outbreaks originating from consumption of raw milk or products made from it (Necidová et al. 2019). The global dairy industry is transforming and it relies heavily on the implementation of strategies to improve and strengthen milk process optimization. Different standards have been laid down by global regulatory agencies for *S. aureus* permissible limits in milk and milk products. For example, as per European Commission, the limit of *S. aureus* in raw milk for drinking and production is <500 cfu/ml and <2000 cfu/ml, respectively (https://ec.europa.eu/food/safety/

international_affairs/trade_en; Hillerton et al. 2004). The permissible limits for *S. aureus* in milk and milk products by various regulatory bodies have been shown in Table 1.

Staphylococcus – general features

Genera *Staphylococcus* refers to Gram positive, cocci-shaped bacteria with characteristic grape-shaped morphology. They are ubiquitous in nature and found in air, water, and soil; but humans and animals serves as primary reservoirs (Hennekinne et al. 2018**)**. The genus is classified into around 53 recognized species and 28 subspecies. *S. aureus*, *S. epidermidis* and *S. saprophyticus* are among the members that are most frequently associated with human infections (PHE-NHS, 2020). Staphylococci are grouped into coagulase and non-coagulase producers. Coagulase negative staphylococci are largely non-pathogenic in nature (Argaw et al. 2015). *S. aureus*, one of the most popular members of *Staphylococcus* genera is a non-motile, non-spore forming, facultative anaerobic, catalase and coagulase producing potential human and animal pathogen having an inherent ability to adhere to epithelial surfaces. It is also among one of the leading causes of bovine mastitis (Sheet et al. 2016). In addition to the coagulase, *S. aureus* produce several heat stable enterotoxins (eg. enterotoxin A) responsible for food poisoning; several cell membrane targeting toxins (alpha, beta, gamma, and delta) (Kashif et al. 2019); and other extracellular proteins, such as, hemolysins, and leukocidins. Classical staphylococcal toxins (SE-A to SE-E) are responsible for more than 90% of *S. aureus* food poisoning outbreaks (Wang et al. 2018). Besides enterotoxins, other toxins like exfoliative toxin A and B, and toxic shock syndrome toxin, are also produced by *S. aureus* (Fagundes and Oliveira, 2004). Further, they possess many surface proteins, which initiate infection by sticking (adhesion phase) to the tissues; and a variety of enzymes, such as proteases, lipases, and hyaluronidases that allow the bacteria to enter (invasion phase) and destroy tissues and spread (evasion phase) to nearby tissues during the infection process (Yaniarti et al. 2017; Maikranz et al. 2020). Heat resistance, toxin production, biofilm formation, antibiotic resistances are among the many characteristics that contribute to pathogenicity of *S. aureus*. In healthy host, *S. aureus* colonizes the nasal mucosa and skin but its opportunistic entry into the bloodstream or internal tissues can cause several lethal infections including hard-to-treat hospital acquired infections (Abril et al. 2020). Presence of lactose phosphotransferase systems, as revealed by genome sequence analysis enables *S. aureus* growth in milk. Staphylococci also requires B vitamins (thiamine and nicotic acid) and inorganic salts for efficient growth. Glutamic acid, leucine, and tyrosine are essential for enterotoxin production, however, are not required for growth (Medvedova and Valik, 2012).

Prevalence and significance of *S. aureus* in dairy sector

Milk chain right from production to processing and storage involving milking animal, animal handlers, milking environment, utensils, industry pipe lines, packaging and storage environment can contribute to entry of *S. aureus* in raw and processed milk. Presence of *S. aureus* in milk chain has considerable impact on overall quality, consumer safety, demand and global supply chain. Animals and animal derived food products have a considerable impact on public health. Animal derived food may be infected with one or more preformed staphylococcal enterotoxins, which can cause human disease (Pal et al. 2020). *S. aureus* can make its way to milk and milk products through air, dust, waste, water, milk, human personnel or animal udders. A large number of factors such as direct usage of raw milk, improper or sub-pasteurization treatment, post-pasteurization contamination, resistance development etc. may contribute to reporting of *S. aureus* in processed milk products. Milk serves as a rich nutrient source for *S. aureus* growth and enterotoxin production. *S. aureus* including multi drug resistant variants are frequently reported in raw milk, processed milk and milk products (Fletcher et al. 2015; Yehia et al. 2019; Zeinhom and Abed, 2021; de Silva Abreu et al. 2021) indicating prevalence of *S. aureus* in milk chain. Frequent reports of *S. aureus* in milk products from across the globe indicate its capability to easily transfer from raw milk to processed milk products.

Staphylococcus enterotoxins related foodborne outbreaks revealed food handling as the most likely contamination source because the isolated *S. aureus* strains were common between food handlers, foods, and/or patient specimens (Johler et al. 2013). Biotyping in combination with phage typing has been proposed to be a useful tool for tracing origin of *S. aureus* strains from food of animal origin (Gill et al. 1994b). Cows suffering from subclinical mastitis are increasingly considered as alternative reservoirs of *S. aureus* leading to contamination of dairy production and processing chain. The *S. aureus* infections in cattle (clinical and subclinical mastitis) are responsible for the reduced milk yield, spoiled milk, poorer milk content, unstable taste, reduced milk processing, lower shelf life, and decreased yield of milk products (Pal et al. 2020). These outcomes lead to huge damage to economy and livestock owners. As recently reported by Giri et al. (2020), mastitis causes nearly 2.37 billion INR losses annually to India's dairy industry. Subclinical mastitis accounted for roughly 70% of the total loss.

S. aureus along with other staphylococci is known to produce biofilms, which contributes to its tolerance to host defense mechanisms and environmental stress. Antimicrobial treatment primarily employed to control *S. aureus* in milking animals contributes to development of antimicrobial resistance in *S. aureus* and other pathogenic strains. *S. aureus* readily acquires resistance to antimicrobials, resulting in persistent non-curable udder infections that often lead to culling of infected animals. Because of its notorious ability to develop resistance to the commonly used as well as last resort antimicrobials and development of multi drug resistant strains, antimicrobial resistance in *S. aureus* is of principal value in human and animal

Table 1 Overview of microbiological criteria/specifications for milk and milk products in context to *Staphylococcus aureus*

Products	FSSR, 2017		FDA Republic of Philippines, 2013		EC, 1992		BIS IS 1165:2002; IS14433:2007; IS 1806: 2018; IS 1656: 2007		FSANZ, 2018	
	m	M	m	M	M	M	M	M	M	M
Skimmed milk powder	-	-	-	-	-	-	-	10/g	-	-
Pasteurized Milk/Flavoured Milk	Methylene Blue Reduction Test (MBRT) applicable at manufacturing unit shall not decolorized in 5 h		-	-	-	-	-		-	-
Pasteurized cream	-	-	-	-	-	-	-		-	-
Sterilized/ UHT/ Flavoured milk/ Evaporated milk	Absent/1g	-	-	-	-	-	-		-	-
Sterilized/ UHT cream	-	-	-	-	-	-	-		-	-
Sweetened condensed milk	<10/g	-	-	-	-	-	-		-	
Butter (Unpasteurized milk) and/or Unpasteurized milk products	Absent/1g	-	-	-	-	-	-		10/g	10^2/g
Pasteurized Butter	10/g	50/g	10^2cfu/ml	-	-	-	-		-	-
Milk powder; SMP, Dairy Whitener; Cream powder; Ice Cream Mix powder; Lactose; Whey based powder; Butter Milk powder; Casein powder	10/g	10^2/g	-	-	10cfu/g	10cfu/g	Absent/0.1g *		-	-
Infant Milk Food, Infant Formulae, Infant Milk Substitute	Absent/0.1g	-	-	-	-	-	Absent/0.1g		-	-
Ice Cream, Frozen Dessert, Milk Lolly, Ice Candy	10/g	10^2/g	10cfu/g	10^2cfu/g	10cfu/g	10cfu/g	-		-	-
Processed Cheese/ Cheese Spread	10/g	-	10cfu/g	10^2cfu/g	-	-	-		-	-
Fresh Cheeses/ Cheddar/ Cottage /Soft, Semi Soft cheese from heat treated milk	10/g	10^2/g	10^2cfu/ml	10^3cfu/g	10^2cfu/g	10^3cfu/g	-		10^2/ml	10^3/ml
Fermented milk products: Yoghurt, Dahi, Chakka, Shrikhand	10/g	10^2/g	10cfu/ml	10^2cfu/ml		-	-		-	-
Paneer/ Chhana/ Chhana based sweets	10/g	10^2/g	-	-	-	-	-		-	-
Khoa/ Khoa based sweets	10/g	10^2/g	-	-	-	-	-		-	-
Malted milk food	-	-	-	-	-	-	Absent/0.1g (with cocoa powder / without cocoa powder)		-	-
Milk cereal based complementary foods/ follow-up formula – complementary food	Absent/0.1g		-	-	-	-	Absent/0.1 g		-	-

m = Represents an acceptable level and values above it are marginally acceptable in terms of the sampling plan. M = A microbiological criterion which indicates unsatisfactory or potentially hazardous quality. Values above M are unacceptable in terms of the sampling plan and detection of one or more samples exceeding this level would be cause for rejection. * = Spray dried milk powder from standardized milk.

FSSR, Food Safety and Standards Regulations; FDA, Food and Drug Administration; EC, European Commission; BIS, Bureau of Indian Standards; IS, Indian Standards; FSANZ, Food Standards Australia New Zealand

medicine (Abdi et al. 2018). Methicillin resistant *Staphylococcus aureus* (MRSA) causes mild to severe infections in humans and animals worldwide (Boswihi and Udo, 2017). Reports of presence of MRSA in foods of animal origin raise concerns about its transmission to humans (Basanisi et al. 2017). Dairy cows having subclinical mastitis can transmit MRSA to milk even without changing the milk organoleptic properties and thus can be linked to its spread to people associated with cattle and milk processing (Basanisi et al. 2017) and also consumers.

Resistance to processing conditions and microenvironment

S. aureus has been shown to survive across wide range of environmental stresses (cold, low water activity (Aw) etc.) and its ability to rapidly adapt to environmental fluctuations determines its pathogenicity (Alreshidi et al. 2015). Each environmental stress is known to influence the expression of several cellular processes, virulence factors and antimicrobial resistant determinants that endows the organism with an ability to survive under stress conditions (Anderson et al. 2006). Thermal tolerance among *S. aureus* is strain specific and varies with many factors. The 'D' value (decimal reduction value) for *S. aureus* has been shown to vary with the media/food matrix in which it is present. Likewise, *S. aureus* was reported to display thermal resistance in order: skim milk > Cheddar cheese whey > phosphate buffer > whole milk (Walker and Harmon, 1966). Recently, Yehia et al. (2019) reported heat resistant *S. aureus* strains from pasteurized camel milk sold in Riyadh City, Saudi Arabia. The enterotoxin producing strain survived heat treatment at 90°C for 2 minutes. Earlier, Montanari et al. (2015) too reported *Staphylococcus sp.* survival at 80°C for 20 minutes. Another study documented that the pasteurization parameters failed to eliminate *S. aureus* from different dairy products. Optimally, 80°C/20 minutes is required to kill *S. aureus* in dairy products (Yaniarti et al. 2017).

S. aureus is resistant to freezing and survives well in food stored below -20°C. Prolonged cold stress induced different metabolomic and proteomic profile at the mid-exponential growth phase of *S. aureus*, compared to those incubated at 37°C. Several (nine) cytoplasmic ribosomal proteins and citric acid were up-regulated in cells adapted to cold-stress. Changes in metabolic homeostasis and protein profile are critical for survival under cold stress (Alreshidi et al. 2015). Pathogens including *S. aureus* possess several additional structural and biochemical features that allow them to resist the host gastrointestinal defense stress (Bera et al. 2005; Panwar et al. 2020). According to recent reports, oral cavity has a high prevalence of *S. aureus* and MRSA (Donkor et al. 2020). Earlier, Vanzato et al. (2010) reported *S. aureus* and MRSA from the oral cavity of healthcare workers. Biofilm forming potential of *S. aureus* may be one of the factors contributing towards prevalence of *S. aureus* in oral cavity.

Staphylococcus sp. has above average pH tolerance and grows over pH range of 4.0-10.0, with an optimum of 6-7. Genes for urease enzyme such as *ure* have been found to be essential for survival under acid stress, which might act by regulating pH homeostasis and urea utilization (Zhou et al. 2019). During acid stress, genes for the arginine deiminase pathway such as *arc*A/B/C becomes activated and lead to concomitant production of ammonia which maintains pH homeostasis and ATP which drives intracellular proton transport (Grosser et al. 2018). Similar function is performed by F_1F_0-ATPase which actively exports protons and regulates pH balance (Pi et al. 2009). Staphylococci also show bile resistance but the mechanisms are not well explored. Recently, *S. aureus* has been shown to have *mnhF* gene, which has a role in providing bile resistance. This operon is known for exchange of protons with Na^+/Li^+ or K^+ and thus *mnhF* gene is supposed to confer bile resistance by active transport of the bile salts (cholate) from *S. aureus* (Vaish et al. 2018).

Staphylococcus species displays complete lysozyme resistance, which helps them persist and successfully colonize the skin and mucosal areas of humans and animals. In *S. aureus*, an integral membrane protein *OatA* codes for O-acetylation at C6-OH of peptidoglycan muramic acid. *OatA* was identified as the molecular basis for high lysozyme resistance in staphylococci (Bera et al. 2005). *S. aureus* is able to survive in potentially dry (dessication resistance) and stressful environments, such as skin, nose, and inanimate surfaces. Chaibenjawong and Foster (2011) identified several genetic determinants (*clpX*, *sigB* and *yjbH*), playing role in dessication tolerance. *S. aureus* being highly osmo-tolerant can thrive well in foods with reduced Aw (Shebuski et al. 2000).

S. aureus is halotolerant and can grow well in the presence of high salt concentrations, such as on skin surfaces which often have high NaCl concentration (10%). Under low salinity, immediate influx of small solutes relieve physical stress; whereas under high salinity, water efflux is counterbalanced by an increase of compatible solutes such as proline, glutamate, glycine betaine, ectoine and trehalose (Omotoyinbo et al. 2017). *S. aureus* can cope with osmotic environments by accumulating osmoprotectants such as, proline and glycine betaine (Hajmeer et al. 2006).

***S. aureus* control in the milk chain**

As discussed and established by scientific data, *S. aureus* is prevalent in dairy environment, animals, animal handlers, raw milk and even makes its way to processed milk and milk products due to its adaptive nature. In order to check *S. aureus* and its enterotoxins in dairy chain, antibiotics are employed which further adds to developing antibiotic resistance. Moreover, available and popular mitigation strategies cannot be applied to milk and milk products without compromising consumer preferences and safety guidelines. Hence, there is a need for natural, safe and cost-effective strategies that can help to minimize *S. aureus* in

milk chain without selecting resistant strains, without targeting commensal flora and without compromising general food sensory characteristics. The section discusses few such emerging promising strategies in brief.

Several probiotic strains have been shown to be effective against mastitis causing pathogens (Assis et al. 2015). Probiotics can also stimulate the immune response in cattle and can modulate internalization of *S. aureus* within the host cells (Zatout et al. 2019). Some *Lactobacillus* spp. strains produce metabolites that prevent *S. aureus* adhesion to udder tissues; resist pathogenicity by producing hydrogen peroxide, altering the host immunity, and competition for nutrients. Sharma et al. (2017) documented the antibacterial effects of *Lactobacillus* isolates of curd and human milk origin against several food-borne and human pathogens. Probiotics and their metabolites can also prevent biofilm formation and dissociate early stage and mature pathogen biofilms. In one such study, *Lactobacillus* spp. strains from goat milk origin showed antagonistic activity against growth and biofilm formation by *Pseudomonas aeruginosa* and *S. aureus* (Singh et al. 2018). Few probiotic based formulations have been recently introduced for management of mastitis in dairy animals. Provilan ANNAA+ Optimum Care spray™, a plant origin probiotic strain formulation have been introduced for preventing mastitis in dairy cows (https:// ingenious-probiotics.com). Another probiotic formulation, Aptamama (*L. salivarius* PS20, recently launched by Danone promises to reduce mastitis incidence amongst healthy lactating mothers by 59% (https:// www.nutraingredients-asia.com).

Phytocompounds have also found to be effective in controlling bovine mastitis causing *S. aureus* isolates (Mordmuang et al. 2019). Due to the presence of phenolic and ethanolic compounds, plant extracts displays effective antioxidant and antimicrobial effects. The combination of these phenolic plant extracts displayed potent bactericidal effect against *S. aureus* biofilms (Gomes et al. 2019). Also, plant ethanolic extracts reduced *S. aureus* internalization into bovine mammary cells (Mordmuang et al. 2019). Active component of *Eucalyptus globulus* has been shown to display potent anti-biofilm and antiquorum sensing activities against MRSA (Merghni et al. 2018). Co-application of phytocompounds and antibiotics can also be an effective strategy against *S. aureus* due to synergistic action of the two. Bacteriophage presents a promising approach for controlling *S. aureus* infection in dairy (Iwano et al. 2018). Phage K can efficiently lyse staphylococci and can be used prophylactically against *S. aureus* infections. Another phage MSA6 is a potential universal agent against *Staphylococcus spp.* (Kwiatek et al. 2012). Nanoparticles have gained attention as effective antimicrobial against *S. aureus* and mastitis in dairy animals. Encapsulation of antibiotics with nanoparticles enhances their activity and targeted site delivery. Lysostaphin, a potential *Staphylococcus* inhibiting enzyme cleaves penta-glycine bridge of *S. aureus* cell wall. Recently, recombinant lysostaphin has been found to cure 95% *S. aureus* udder infection (Aqib et al. 2021).

Vaccination is used as a powerful strategy for prevention and even eradication of infectious diseases; besides restraining MDR bacteria including *S. aureus*. The strategy is promising as a valid alternative therapeutic to antibiotics in animals and humans; besides possessing an added advantage of being free from resistance development (Sharma et al. 2018). Vaccines turn out to be the most effective measure to prevent bovine mastitis. Commercially available vaccines against *S. aureus* include Lysigin® and Starvac®. Besides the above mentioned strategies, proper precautions like maintenance of hygiene during milking and other processing steps can considerably reduce *S. aureus* entry in milk and milk products.

S. aureus – traditional and rapid identification approaches

Identification and characterization of *S. aureus* is primarily carried out on basis of characteristic morphological and biochemical properties during its culture. Isolation of staphylococci from milk involves initial enrichment in common enrichment media (eg. Giolotti-Cantoni Broth, Tryptic Soy Broth) followed by plating over selective and differential media (eg. Baird Parkar). Candidate staphylococci displaying typical colony characteristics over differential media are subjected to staining for morphological identification as non-motile, Gram positive cocci with grape like cluster arrangement. Biochemical identification is primarily based on catalase, and coagulase production, agglutination tests (clumping factor, protein A), and sugar fermentation tests that besides identifying *S. aureus*, also differentiates it from other closely related staphylococcal species. *S. aureus* gives positive reaction for catalase, citrate, urease, coagulase production, lipid hydrolysis, and mannitol fermentation; and have thermostable deoxyribonuclease (Tang and Stratton 2010).

Rapid detection of any potential food pathogen with high sensitivity and reproducibility is of significance for ensuring food quality and safety. Polymerase Chain Reaction (PCR) methods (PCR, Real Time Quantitative PCR) offers ability to rapidly identify *S. aureus* on basis of unique DNA sequences targeted *via* single-plex and multi-plex PCR reactions. In recent years, Loop mediated isothermal amplification (LAMP) assay has emerged as a simple, rapid and cost-effective DNA based tool for detection of pathogens. The results of LAMP assay can be visualized by naked eyes (Tian et al. 2018). Although the DNA based identification is quick, sensitive and reproducible; it requires skills, high-end infrastructure; besides need for enrichment, growth in liquid or solid media, and selection of pure colonies. This is followed by DNA isolation, PCR amplification and electrophoresis (not required for qPCR) steps, which slows the rapid identification, especially when it comes to a perishable commodity, such as milk. Some other rapid identification strategies have been

proposed and are likely to have application in early detection of *S. aureus* in milk.

Recently, a one-step enzyme free, label free, fluorometric strategy (Target Inhibited Fluorescence Signal Recovery) based on nanometal surface energy transfer between carbon dots and gold nanoparticles (AuNPs) has been developed for facile detection of *S. aureus*. The proposed strategy has enhanced detection limit (10 cfu/mL) for *S. aureus* (Yao et al. 2021). Hu et al. (2021) developed a Nanobodies (Nbs) sandwich ELISA based immunoassay for screening of *S. aureus*. The detection ability was verified by detection of 10 cells per ml of *S. aureus* in 8hr enriched milk samples.

Phage endolysins contains N-terminal catalytic domain and C-terminal cell wall binding domains. Lysin cell wall binding domains (CBD) are substrate specific and bind bacterial cell wall receptors by non-covalent bonds. In a recent study, phage lysin cell wall binding domains along with immunomagnetic particles were used for *S. aureus* detection in milk (Yu et al. 2016). M13 phage has also been explored for early pathogen detection. In one such study, surface enhanced Raman scattering gold nanoprobe based on pIII protein of M13 phage was used for early detection and de-activation of *S. aureus*. The probe could quantify *S. aureus* loads within range of 10-10^6 cells/mL. Furthermore, this probe has antibacterial potential towards *S. aureus* (Wang et al. 2021).

Recently, a new strategy using modified propidium monoazide (PMA) dye combined with recombinase aided amplification (RAA) was proposed for the rapid and real-time detection of viable *S. aureus* in milk. Xie and co-workers (2021) developed a PMAxx-RAA combination in a microplate assay for *S. aureus* detection in milk. Following enrichment, limit of detection for viable *S. aureus* ranged from 10^2cfu/mL (3 h enrichment) to 10^1cfu/mL (6 h enrichment) in spiked milk samples (Xie et al. 2021). Liu et al. (2021) standardized a flow cytometry based method for *S. aureus* detection in milk and milk powder. In this methodology, fluorescently labeled antibodies and propidium iodide were used for selective detection of viable *S. aureus*. Following a 5 hour enrichment period, the method could detect around 7 *S. aureus* cells/mL in milk. In another approach, Yang et al. (2021) developed magnetic apatamer biosensor based on personal glucose meter for food pathogen detection. The biosensor could detect *S. aureus* with least detection count of 2 cells/mL. Another biosensor based approach involved design of an electrochemical biosensor based on triple helix molecular switch for detection of various pathogens in food and environment. This electrochemical biosensor can broadly detect *S. aureus* within dynamic range from 30 to 3×10^8 CFU/mL and having least detection count of 8 cell per unit sample (Cai et al. 2021).

Conclusions

Staphylococcus aureus has diverse metabolic potential and it often causes diseases in both animal and humans. *S. aureus* resides in different host body parts and food types, but one of its important sources is milk, which is widely consumed, and thus *S. aureus* becomes an important food pathogen. It manifests disease through its toxins which vary in their nature and action depending and they frequently have mechanisms to either evade or overwhelm the host immune system. Additionally the pathogen is resistant to different types of external stress conditions and thus spoils a range of food items including the processed ones. Due to its genomic plasticity and multiple drug resistance, it features in WHO's top list of microbes for which antibiotics are urgently needed. Recently, non-antibiotic interventions are being explored as alternative strategies against it, which are yet to become pharmacological practice. High throughput, on-site rapid detection of *S. aureus* present in low cell counts in milk is also very important to prevent food poisoning outbreaks and other clinical conditions. Multi-dimensional understanding of *S. aureus* pathology, reservoir-transmission dynamics and rapid detection would help in careful mitigation of the issue of *S. aureus* inside hosts and in overall dairy sector.

References

Abdi RD, Gillespie BE, Vaughn J, Merrill C, Headrick SI, Ensermu DB, D'Souza DH, Agga GE, Almeida RA, Oliver SP, Dego OK (2018) Antimicrobial resistance of *Staphylococcus aureus* isolates from dairy cows and genetic diversity of resistant isolates. Foodborne Pathog Dis 15: 449-458

Abril AG, Villa, TG, Barros-Velázquez J, Cañas B, Sánchez-Pérez A, Calo-Mata P, Carrera M (2020) *Staphylococcus aureus* exotoxins and their detection in the dairy industry and mastitis. Toxins 12: 537

Alreshidi MM, Dunstan RH, Macdonald MM, Smith ND, Gottfries J, Roberts TK (2015) Metabolomic and proteomic responses of *Staphylococcus aureus* to prolonged cold stress. J Proteomics 121: 44-55

Anderson KL, Roberts C, Disz T, Vonstein V, Hwang K, Overbeek R, Olson PD, Projan SJ, Dunman PM (2006) Characterization of the *Staphylococcus aureus* heat shock, cold shock, stringent, and SOS responses and their effects on Log-phase mRNA turnover. J Bacteriol 188: 6739-6756

Aqib AI, Ijaz M, Shoaib M, Muzammil I, Hussain HI, Zaheer T, Naseer MA (2021) *Staphylococcus aureus* and dairy udder. In *Staphylococcus aureus*. Intech Open. Doi: 10.5772/intechopen.95864

Argaw S, Addis M (2015) A review on staphylococcal food poisoning. Food Sci Quality Management 40: 59-71

Assis BS, Germon P, Silva AM, Even S, Nicoli JR, Le Loir Y (2015) *Lactococcus lactis* V7 inhibits the cell invasion of bovine mammary epithelial cells by *Escherichia coli* and *Staphylococcus aureus*. Benef Microbes 6: 879-886

Basanisi MG, La Bella G, Nobili G, Franconieri I, La Salandra G (2017) Genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and dairy products in South Italy. Food Microbiol 62: 141-6

Bera A, Herbert S, Jakob A, Vollmer W, Gotz F (2005) Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan O-

acetyltransferase OatA is the major determinant for lysozyme resistance of *Staphylococcus aureus*. Mol Microbiol 55: 778-787

Boswihi SS, Udo EE (2018) Methicillin-resistant *Staphylococcus aureus*: an update on the epidemiology, treatment options and infection control. Curr Med Res Pract 8: 18-24

Cai R, Zhang Z, Chen H, Tian Y, Zhou N (2021) A versatile signal-on electrochemical biosensor for *Staphylococcus aureus* based on triple-helix molecular switch. Sens. Actuators B Chem 326: 128842

Chaibenjawong P, Foster SJ (2011) Desiccation tolerance in *Staphylococcus aureus*. Arch Microbiol 193: 125-135

da Silva Abreu AC, Matos LG, da Silva Cândido TJ, Barboza GR, de Souza VVMA, Nuñez KVM, Silva NCC (2021) Antimicrobial resistance of *Staphylococcus* spp. isolated from organic and conventional Minas Frescal cheese producers in São Paulo, Brazil. J Dairy Sci 104: 4012-4022

Donkor ES, Kotey FC (2020) Methicillin-Resistant *Staphylococcus aureus* in the Oral Cavity: Implications for antibiotic prophylaxis and surveillance. Infectious Dis Res Treatment 13: 117863372097658

EC (European Commission) (1992) Council Directive 92/46/EEC. Laying down the health rules for the production and placing on the market of raw milk, heat treated milk and milk based products. Online. Available at https://eur-lex.europa.eu/legal content/EN/TXT/PDF/?uri=CELEX:31992L0046&from=EN Accessed March 26, 2021.

Fagundes H Oliveira CAF (2004) *Staphylococcus aureus* intra-mammary infections and its implications in public health. Cienc Rural 34: 1315-1320

FDA, Philippines (2013) Revised guidelines for the assessment of microbiological quality of processed foods. Department of Health, Food and Drug Administration, FDA circular no. 2013-010. Online available at Accessed March 27, 2021

Fletcher S, Boonwaat L, Moore T, Chavada R, Conaty S (2015) Investigating an outbreak of staphylococcal food poisoning among travellers across two Australian states. Western Pac Surveill Research J 6: 17

FSANZ (Food Standards Australia New Zealand (2018) In. Compendium for microbiological criteria for food-Dairy Products 32-38 Online available at https://www.foodstandards.gov.au/publications/Documents/Compedium%20of%20Micrbiological%20Criteria/Compendium_revised-Sep%202018.pdf Accessed March 27, 2021

FSSR (2017) Revised standards for milk and milk products. In The Gazette of India, extraordinary Part-III, section 4 Published by Food Safety and Standards Authority of India on October 12, 2017. Online available at https://fssai.gov.in/upload/uploadfiles/files/Gazette_Notification_Milk_Products_24_10 2017.pdf. Accessed March 27, 2021

Fusco V, Chieffi D, Fanelli F, Logrieco AF, Cho GS, Kabisch J, Bohnlein C, Franz CM (2020) Microbial quality and safety of milk and milk products in the 21st century. Comprehensive Rev Food Sci Food Safety 19: 2013-2049

Gill JPS, Joshi DV and Kwatra MS (1994a) Qualitative bacteriological survey of milk and milk products with special reference to *Staphylococcus aureus*. J Dairy Sci 47: 8

Gill JPS, Joshi DV and Kwatra MS (1994b) Biotyping of *Staphylococcus aureus* strain isolated from food of animal origin. Indian J Appl Res 64: 668-671

Giri AK, Shrman K, Jamra MS, Prajapati BK (2020) A review on mastitis in dairy animals. Int J Curr Microbiol App Sci 9: 1844-1852

Girma K, Tilahun Z, Haimanot D (2014) Review on milk safety with emphasis on its public health. World J Dairy Food Sci. 9:166-83

Gomes F, Martins N, Ferreira IC, Henriques M (2019) Anti-biofilm activity of hydromethanolic plant extracts against *Staphylococcus aureus* isolates from bovine mastitis. Heliyon 5: e01728

Grosser MR, Elyse P, Thurlow LR, Dillon MM, Cooper VS, Kawula TH, Richardson AR (2018) Genetic requirements for *Staphylococcus aureus* nitric oxide resistance and virulence. PLOS Pathog 14:e1006907

Hennekinne JA (2018) *Staphylococcus aureus* as a Leading Cause of foodborne outbreaks worldwide. In. *Staphylococcus aureus,* Academic Press Publisher 129-146

Hillerton JE, Berry EA (2004) Quality of the milk supply: European regulations versus practice. In NMC Annual Meeting Proceedings 207: 214

Hu Y, Sun Y, Gu J, Yang F, Wu S, Zhang C, Wang S (2021) Selection of specific nanobodies to develop an immuno-assay detecting *Staphylococcus aureus* in milk. Food Chem 129481

IS 1165: 2002, BIS (Bureau of Indian Standards). Specification for milk powder. Fifth revision reaffirmed in 2009, 2012 and 2018 New Delhi, India

IS 14433: 2007, BIS (Bureau of Indian Standards). Specification for infant milk substitute. First revision reaffirmed in 2012 and 2018 New Delhi, India

IS 1656: 2007, BIS (Bureau of Indian Standards). Specification for milk cereal based complementary foods. Fourth revision reaffirmed in 2009, 2012 and 2018 New Delhi, India

IS 1806: 2018, BIS (Bureau of Indian Standards). Specification for malted milk food. Second revision New Delhi, India

Iwano H, Inoue Y, Takasago T, Kobayashi H, Furusawa T, Taniguchi K, Fujiki J, Yokota H, Usui M, Tanji Y, Hagiwara K, Higuchi H, Tamura Y (2018) Bacteriophage ÖSA012 has a broad host range against *Staphylococcus aureus* and effective lytic capacity in a mouse mastitis model. Biology 7: 8

Johler S, Tichaczek-Dischinger PS, Rau J, Sihto HM, Lehner A, Adam M, Stephan R. (2013) Outbreak of Staphylococcal food poisoning due to SEA-producing *Staphylococcus aureus*. Foodborne Patho Dis 10: 777-781

Kashif A, McClure JA, Lakhundi S, Pham M, Chen S, Conly JM, Zhang, K (2019) *Staphylococcus aureus* ST398 Virulence is associated with factors carried on prophage ²”Sa3. Front Microbio 10: 2219

Kwiatek M, Parasion S, Mizak L, Gryko, R, Bartoszcze, M, Kocik J (2012) Characterization of a bacteriophage, isolated from a cow with mastitis, that is lytic against *Staphylococcus aureus* strains. Arch Virol 157: 225-234

Liu S, Wang B, Sui Z, Wang Z, Li L, Zhen X, Zhou G (2021) Faster Detection of *Staphylococcus aureus* in milk and milk powder by flow cytometry. Foodborne Patho Dis doi.org/10.1089/fpd.2020.2894

Medveïová A, Valík ¼ (2012) *Staphylococcus aureus*: Characterisation and quantitative growth description in milk and artisanal raw milk cheese production. In-Tech, 4: 71-102 Doi: 10.5772/48175

Merghni A, Noumi E, Haddad O, Dridi N, Panwar H, Ceylan O, Mastouri M, Mejd S (2018) Assessment of the antibiofilm and antiquorum sensing activities of Eucalyptus globules essential oil and its main component 1,8-cineole against methicillin-resistant *Staphylococcus aureus* strains. Microbial Pathogenesis 118: 74-80

Montanari C, Serrazanetti DI, Felis G, Torriani S, Tabanelli G, Lanciotti R, Gardini F (2015) New insights in thermal resistance of staphylococcal strains belonging to the species *Staphylococcus epidermidis*, *Staphylococcus lugdunensis* and *Staphylococcus aureus*. Food Control 50: 605-612

Mordmuang A, Brouillette E, Voravuthikunchai SP, Malouin F (2019) Evaluation of a Rhodomyrtus tomentosa ethanolic extract for its

therapeutic potential on *Staphylococcus aureus* infections using *in-vitro* and *in-vivo* models of mastitis. Vet Res 50: 1-11

Necidová L, Bursová Š, Haruštiaková, D, Bogdanovièová K, Laèanin I (2019) Effect of heat treatment on activity of staphylococcal enterotoxins of type A, B, and C in milk. J Dairy Sci 102: 3924-3932

Omotoyinbo OV, Omotoyinbo BI (2017) Effect of Varying NaCl Concentrations on the growth curve of *Escherichia coli* and *Staphylococcus aureus* Cell Biol 4: 31-34

Pal M, Kerorsa GB, Marami LM, Kandi V (2020) Epidemiology, pathogenicity, animal infections, antibiotic resistance, public health significance, and economic impact of *Staphylococcus aureus*: a comprehensive review. Am J Public Health 8: 14-21

Panwar H, Rokana N, Aparna SV, Kaur J, Singh A, Singh J, Puniya AK (2020) Gastrointestinal stress as innate defense against microbial attack. J Appl Microbiol 130: 1035-1061

Pi B, Yu M, Chen Y, Yu Y, Li L (2009) Distribution of the ACME-ArcA gene among meticillin resistant *Staphylococcus haemolyticus* and identification of a novel Ccr allotype in ACME-ArcA-positive isolates. J Med Microbiol 58: 731–36

Procopio TF, Moura MC, Bento EFL, Soares T, Coelho LCBB, Bezerra RP, Mota RA, Porto ALF, Paiva PMG, Napoleão TH (2019) Looking for alternative treatments for bovine and caprine mastitis: Evaluation of the potential of *Calliandra surinamensis* leaf pinnulae lectin (CasuL), both alone and in combination with antibiotics. Open Microbiol J 8: e809

Public Health England (PHE)-National Health Service (NHS) (2020) UK standards for microbiology investigations Identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species. 07: 1-26

Sharma C, Rokana N, Chandra M, Singh BP, Gulhane RD, Gill J PS, Panwar H (2018) Antimicrobial resistance: its surveillance, impact, and alternative management strategies in dairy animals. Front Vet Sci 4: 237

Sharma C, Singh BP, Thakur N, Gulati S, Gupta S, Mishra SK, Panwar H (2017) Antibacterial effects of Lactobacillus isolates of curd and human milk origin against food-borne and human pathogens. 3 Biotech 7:31

Shebuski JR, Vilhelmsson O, Miller KJ (2000) Effects of growth at low water activity on the thermal tolerance of *Staphylococcus aureus*. J Food Prot 63: 1277-1281

Sheet OH, Grabowki NT, Klein G, Abdulmawjood A et al (2016) Development and validation of a loop mediated isothermal amplification (LAMP) assay for the detection of *Staphylococcus aureus* in bovine mastitis milk samples. Mol Cell Probes 30: 320-325

Singh N, Sharma C, Gulhane RD, Rokana N, Singh BP, Puniya AK (2018) Inhibitory effects of lactobacilli of goat's milk origin against growth and biofilm formation by pathogens: An in vitro study. Food Biosci 22: 129-138

Tang YW, Stratton CW (2010) *Staphylococcus aureus*: An old pathogen with new weapons. Clin Lab Med 30: 179-208

Tian X, Feng J, Wang Y (2018) Direct loop-mediated isothermal amplification assay for on-site detection of *Staphylococcus aureus*. FEMS Microbiol Lett 365(11): fny092. https://doi.org/10.1093/femsle/fny092

Vaish M, Price-Whelan A, Reyes-Robles T, Liu J, Jereen A, Christie S, Alonzo F et al (2018) Roles of *Staphylococcus aureus* Mnh1 and Mnh2 antiporters in salt tolerance, alkali tolerance, and pathogenesis. J Bacteriol 200:1-15

Vanzato PI, Gir E, Pimenta FC et al (2010) Does the oral cavity represent an important reservoir for MRSA in healthcare workers? J Hosp Infect 76: 277-278

Walker GC, Harmon LG (1966) Thermal Resistance of *Staphylococcus aureus* in milk, whey, and phosphate buffer. Appl Microbiol 14: 584–590

Wang W, Lin X, Jiang T, Peng Z, Xu J, Yi L, Baloch Z (2018) Prevalence and characterization of *Staphylococcus aureus* cultured from raw milk taken from dairy Cows with mastitis in Beijing, China. Front Microbio 9: 1123

Wang XY, Yang JY, Wang YT, Zhang HC, Chen ML, Yang T, Wang JH (2021) M13 phage based nanoprobe for SERS detection and inactivation of *Staphylococcus aureus*. Talanta 221:121668

Xie G, Zhou D, Zhao G, Feng X, Aguilar Z P, Xu H (2021) Recombinase aided amplification with photoreactive DNA-binding dye for rapid detection of viable *Staphylococcus aureus*. LWT-Food Sci Technol135: 110249

Xihong Z, Li Y, Park M, Wang J, Zhang Y, He X, Forghani F, Wang L et al (2013) Loop mediated isothermal amplification assay targeting the fem A gene for rapid detection of *Staphylococcus aureus* from clinical and food samples. J Microbiol Biotechnol 23: 246-250

Yang Y, Wu T, Xu LP, Zhang X (2021) Portable detection of *Staphylococcus aureus* using personal glucose meter based on hybridization chain reaction strategy. Talanta 226:122132

Yaniarti MN, Amarantini C, Budiarso TY (2017) The effect of temperature and pasteurization time on *Staphylococcus aureus* isolates from dairy products. 8th International Conference on Global Resource Conservation (ICGRC 2017) AIP Conf

Yao S, Zhao C, Shang M, Li J, Wang J (2021) Enzyme-free and label-free detection of *Staphylococcus aureus* based on target-inhibited fluorescence signal recovery. Food Chem Toxicol 150: 112071

Yehia HM, Ismail EA, Hassan ZK, Al-masoud AH, Al-Dagal MM (2019) Heat resistance and presence of genes encoding staphylococcal enterotoxins evaluated by multiplex-PCR of *Staphylococcus aureus* isolated from pasteurized camel milk. Bio Sci Rep 39: BSR20191225

Yu J, Zhang Y, Zhang Y, Li H, Yang H, Wei H (2016) Sensitive and rapid detection of *Staphylococcus aureus* in milk *via* cell binding domain of lysin. Biosens Bioelectron 77: 366–371

Zeinhom M, Abed A (2021) Prevalence, characterization, and control of *Staphylococcus aureus* isolated from raw milk and Egyptian soft cheese. J Vet Med Res 27: 152-160

Zhou C, Bhinderwala F, Lehman MK, Thomas VC, Chaudhari SS, Yamada KJ, Foster KW, Powers R, Kielian T, Fey PD(2019) Urease is an essential component of the acid response network of *Staphylococcus aureus* and is required for a persistent murine kidney infection. PLoS Patho 15: e1007538

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RESEARCH ARTICLE

Fennel (*Foeniculum vulgare*) and Ajwain (*Trachyspermum ammi*) extracts as potential preservatives in processed cheese foods

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Abstract: The study was conducted to evaluate the possibility of utilization of fennel and ajwain extracts as natural preservatives in processed cheese foods. Processed cheese foods were prepared with non dairy ingredients of potato, peanuts and inulin separately and treated with fennel and ajwain extracts. The level of extract addition was optimized to 1.2% total solids and based on sensory liking, fennel extract was added into potato and inulin added cheeses and ajwain extract was added to peanut added cheese. The effect of these extracts on the sensory, physico-chemical and microbiological characteristics of processed cheese foods during storage under refrigerated (7-8 °C) conditions was evaluated. No significant ($p > 0.05$) change was observed in the sensory parameters of all the samples during storage, however the flavour was better maintained in extract added cheeses. A signiûcant ($p < 0.05$) effect was observed on the tyrosine content and free fatty acids as the extract added cheeses exhibited signiûcantly ($p < 0.05$) lower tyrosine and free fatty acid values. Electrophoretic study indicated lower protein degradation in spice extract added samples compared to those without extracts. A signiûcant ($p < 0.05$) effect was also observed on the microbiological characteristics of the products as treated samples showed signiûcantly ($p < 0.05$) lower values for yeast and mold count. Coliforms were not detected throughout the storage period. Fennel and ajwain extracts successfully improved the storage quality of processed cheese foods and may be commercially exploited as natural preservatives in cheese foods.

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Introduction

Processed cheese (PC) is defined as the product produced by heating mixture of various cheese types with different degrees of ripening in the presence of appropriate emulsifying salts (Salek et al. 2015). Processed cheese foods (PCF) are prepared by adding non-dairy ingredients to the mixture (Guinee et al. 2004; Kapoor & Metzger, 2008). Addition of non-dairy ingredients into the processed cheese might affect its keeping quality. To meet the growing market demand for cheese and cheese products it is important for the cheese-making industries to ensure the microbiological safety and stability of these products (Cusato et al. 2013; Dias et al. 2012). Therefore, to make the product shelf stable, preservatives are added. Nowadays, because of the consumer preferences for natural foods, an increased interest has been directed towards plant-based extracts as antimicrobials. Spices have been added as antimicrobials in many foods since ancient times. While substantial data exist in favour of the use of cardamom, cinnamon, clove and others as natural antimicrobials (Gyawali and Ibrahim, 2012; Hayek et al. 2013; Tajkarimi et al. 2010), interest in the antimicrobial properties of ajwain and fennel has recently emerged.

Fennel (*Foeniculum vulgare)* belongs to family *Apiaceae*. It is an indigenous herb of the Mediterranean Sea and seeds are used as spice. It contains essential oil, phenylpropanoids, monoterpenids, sesquiterpenes, coumarins, triterpenoids, tannins, flavonoids, cardiac glycosides, saponins and other types of compounds (He and Huang, 2011). Fennel seed extracts are known to possess antimicrobial activity (Abed 2007) and are reported to inhibit the growth of *Curvularia lunata, Fusarium oxysporum, A. Alternate*, *Mucor roxuii*, *Bacillus cereus, Clostridium botulinum, Salmonella enteritidis, Staphylococcus aureus, Yersinia enterocolitica* (Thakur et al. 2013; Prabha et al. 2002; Ceylan and Fung, 2004). Ajwain (*Trachyspermum ammi*) also belongs to the *Apiaceae* family. It is a highly valued and medicinally important seed-spice grown in Iran, India, Pakistan and Egypt. It is commonly used as a spice and a traditional medicine in India. It is reported to have antifungal/ antibacterial

effects due to the presence of phenolic compounds thymol and carvacrol in it (Morsi 2000, Nagalakshmi et al. 2000). While the antimicrobial activity of thyme, rosemary, cinnamon, sage, cumin, clove and oregano has been reported in dairy products (Tajkarimi et al. 2010), the effect of fennel and ajwain seeds is not reported anywhere. Therefore, the objective of this study was to evaluate the effect of aqueous extracts of fennel and ajwain seeds on the shelf life of PCF.

Materials and Methods

Materials

Cheddar cheese was obtained from the dairy plant of NDRI Bengaluru. Ingredients like potatoes, peanuts, fennel, ajwain and common salt were purchased from the local market. Inulin (Frutafit HD) was obtained from M/s DKSH India Pvt. Ltd., Bengaluru. Tri-sodium citrate was used as emulsifying salt for PC preparation.

Preparation of the extracts

Fennel and ajwain seeds (250 g each) were soaked separately overnight in 200 mL water and ground in a grinder. During grinding additional 50-100 mL water was added and the contents were filtered through muslin cloth. The extracts obtained were stored in the refrigerator till its use.

Manufacture of processed cheese foods

Processed cheese and processed cheese foods were prepared according to the standardized procedure (Rafiq & Ghosh, 2017). The prepared spice extracts were added at a level of 1.2% total solids at the end of processing so that the antimicrobial properties are not affected due to heat treatment. Based on the preliminary trial likings, fennel extract was added into the potato and inulin incorporated cheese whereas ajwain extract was added into the peanut incorporated processed cheese. The cheeses were hot packed in polypropylene cups of 150 g capacity, covered with aluminium laminates and kept at room temperature to cool. The cups were heat sealed after 2 hours and stored at 7-8ºC for storage study. Samples were drawn randomly at intervals of 10 days for physico-chemical, sensory and microbiological evaluations. The samples were coded as CPC- Control processed cheese, PO- Potato incorporated processed cheese, PE- Peanut incorporated processed cheese, IN- Inulin incorporated processed cheese, POF- Potato incorporated processed cheese with fennel extract, PEA- Peanut incorporated processed cheese with ajwain extract, INF- Inulin incorporated processed cheese with fennel extract.

Sensory evaluation

Sensory analysis was performed on a 20-point score card according to the methodology described by Meyer (1973). Cheese slices (about 2 cm to 3 cm) of 10 g to 15 g of each were presented in a covered glass petri plate in a random order with coded numbers. The judged parameters were: appearance (4), body and texture (8) and flavour (8). The evaluation was carried out under proper lighting by an expert panel of minimum eight judges.

Physico-chemical analysis

pH

Grated processed cheese (20 g) was macerated into slurry using 20 mL distilled water at 40°C. pH was measured directly by inserting the electrode into the slurry (Awad et al. 2005).

Free fatty acids

The free fatty acid content of the processed cheese samples was determined using BDI reagent (Deeth & Fitz-gerald, 1976). One measure of free fatty acid is also expressed as acid degree value (Hydrolytic rancidity). Therefore, FFA/ADV is defined as the milli-equivalent of alkali (KOH) required to neutralize the free fatty acids in 100 g of fat.

Tyrosine value

The proteolysis of cheese was measured in terms of soluble tyrosine by Hull methods described by Samples et al. (1984).

UREA Polyacrylamide Gel Electrophoresis (PAGE)

The protein degradation in processed cheese was measured by alkaline UREA- PAGE as per the method described by Creamer (1991).

Microbiological analysis

The microbial analyses were carried out using the methods described by Houghtby et al. (1993). Eleven grams of sample was taken in a pre-sterilized pestle and mortar mixed properly with 99 ml of 0.1% saline. Further dilution to the desired level was carried out by serially transferring 1 ml of diluted sample to 9 ml sterile saline.

Yeast and Mould count

Yeast and Mould counts were determined by plating 1, 2 dilutions of processed cheese using Potato Dextrose Agar (Hi-Media). The pH of the medium was adjusted to around 3.5 by adding 1 ml of sterile tartaric acid solution (10%) to 100 ml of sterilized media, before pouring the media into plates. The count was taken after 3 days of incubation at 30ºC.

Coliform count

Coiform count was determined by plating 1ml of product suspension employing Violet Red Bile Agar (Hi-Media). Plates were incubated at 37°C for 24-48 hours in duplicates. Colonies

with dark red coloration were counted and expressed as cfu/g of the product.

Statistical analysis

The data was compiled and analyzed using the statistical software SPSS 16.0 (Stat Soft Polska, Poland). One way analysis of variance (ANOVA) was performed and significant differences among samples were reported according to Duncan's test at $p < 0.05$.

Results and Discussion

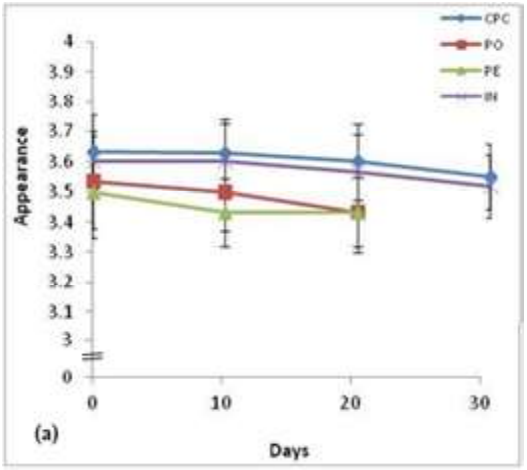
Sensory evaluation

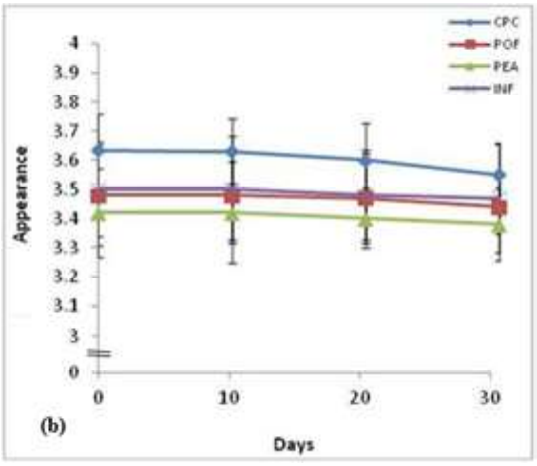
The changes in the sensory parameters during storage are depicted in Figure 1. Visible mould growth was observed on the surface of PO and PE on 30th day of storage hence were discontinued and not served for sensory evaluation and the analysis was carried out till 20th day of storage. The initial appearance scores of spice extract added samples were lower than the samples without spice extracts and control cheese and followed the same trend throughout the storage. The lower appearance scores were due to the addition of fennel and ajwain

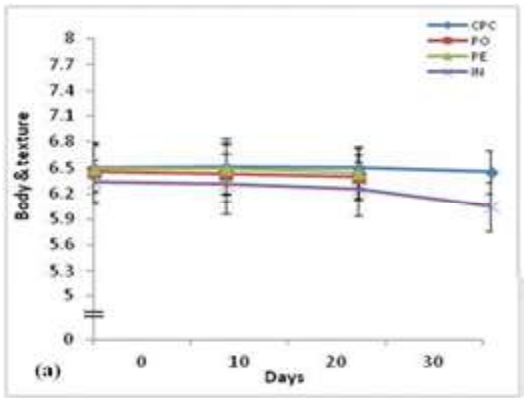
Table 1 Compositional analysis of processed cheese and processed cheese foods

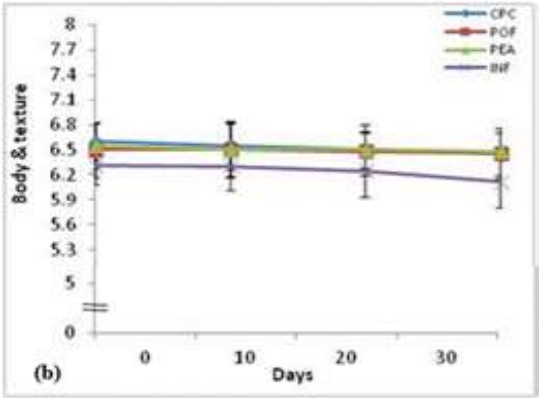
	SAMPLES	Moisture (%)	Total Fat (%) Protein (%)	Total Ash (%)	Total Carbohydrate (%)
CPC	42.19 ±1.03^{a}	30.08±0.48^{b}	22.58±0.74^{a}	4.32±0.12^{a}	0.58±0.05^{d}
PO	44.39±0.86^{a}	23.74±1.05^{d}	18.65±0.35^{c}	3.65±0.25^{b}	8.74±0.94^{a}
PE	42.13 ±0.63^{a}	31.82 ±0.72^{a}	20.96±0.88^{a}	3.74±0.23^{b}	2.08±0.42^{c}
IN	39.61±0.71^{ab}	27.09±0.55^{c}	20.34±00.49^{c}	3.89± 0.14^{b}	6.94±0.32^{b}
POF	44.07±0.78^{a}	22.44±0.85^{d}	19.37±0.55^{c}	3.53±0.22^{b}	8.23±0.48^{a}
PEA	39.96±0.53^{ab}	31.26 ±0.44^{a}	20.78±0.72^{a}	3.63±0.33^{b}	2.53±0.32^{c}
INF	38.49±0.67^{b}	27.35±0.72^{c}	19.75±00.83^{c}	3.72±0.28^{b}	7.14±0.45^{b}

The results are expressed as mean± standard deviation (n = 6); means with different superscripts in a row differ significantly ($p<0.05$). CPC-Control processed cheese, PO- Potato incorporated processed cheese, PE- Peanut incorporated processed cheese, IN- Inulin incorporated processed cheese, POF- Potato incorporated processed cheese with fennel extract, PEA- Peanut incorporated processed cheese with ajwain extract, INF- Inulin incorporated processed cheese with fennel extract.









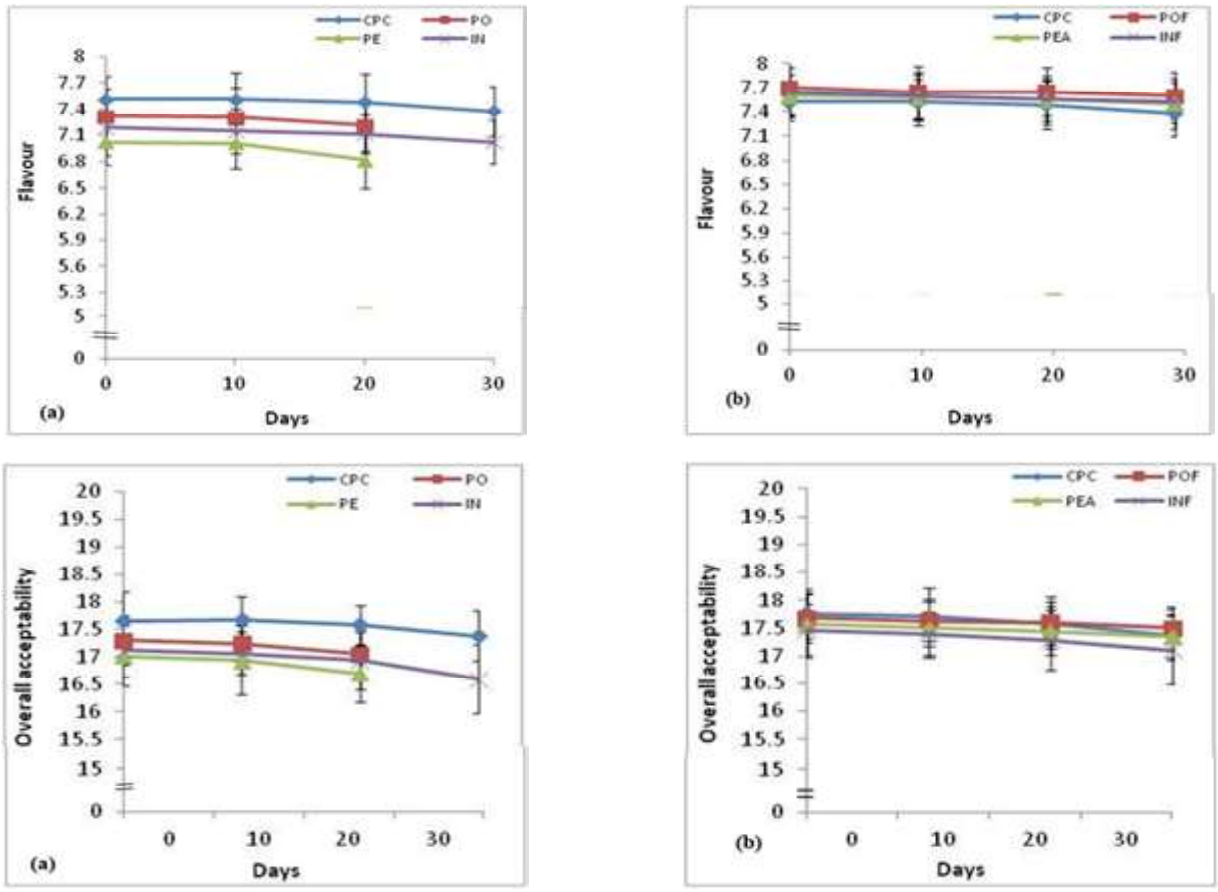


Fig. 1 Effect of non dairy ingredients on: (A) appearance, (B) body &texture, (C) flavour and (D) overall acceptability of processed cheese during storage; (a) Without spice extracts, (b) With spice extracts; CPC- Control processed cheese, PO- Potato incorporated processed cheese, PE- Peanut incorporated processed cheese, IN- Inulin incorporated processed cheese, POF- Potato incorporated processed cheese with fennel extract, PEA- Peanut incorporated processed cheese with ajwain extract, INF- Inulin incorporated processed cheese with fennel extract.

extracts which imparted slight green and brown colour respectively to the treated cheese samples. However, during storage no significant change ($p > 0.05$) was observed in appearance score among the cheese samples irrespective of treatments. The initial body and texture scores were lowest in IN both with and without fennel extract compared to control and other treated samples however, no significant difference ($p > 0.05$) was found among the samples. The lowest scores in IN might be because inulin which is a soluble fibre resulted in decrease in moisture and fat content (Table 1) and the cheese appeared to be dry and firm compared to other samples and was not liked much by the panellists. As the storage progressed the body and texture scores decreased in all the samples but no significant difference ($p > 0.05$) was observed between the samples.

The initial flavour scores of PCFs were slightly lower than the control. It was observed that during storage there was no significant change ($p > 0.05$) in the flavour score of all the cheese samples irrespective of different treatments. However, at 20th day of storage, a slight decrease was observed in the flavour score of PO and PE compared to control. The decrease in the flavour scores of potato and peanut incorporated cheese might be due to the increased proteolysis and lipolysis as evident from Figure 2B and 2C which produced slight acid and rancid flavours respectively. This could be due to the increased microbial activity in these samples because of high carbohydrate and moisture content (Table 1) providing favourable condition for the rapid microbial activity. Addition of spice extracts into PCFs improved the flavour and resultant cheeses showed higher initial flavour scores than those without spice extracts. During storage no significant change ($p > 0.05$) was observed in the flavour score of spice extract added cheese samples compared to those without spice extracts and control. Moreover, the flavour was better maintained during storage contributing to its acceptability. The overall acceptability scores of the cheese samples with spice extracts were higher as compared to those without any added extracts both initially as well as towards the end of storage

however, no significant difference ($p > 0.05$) was found among the samples. The higher acceptability scores might be because of the higher flavour scores of extract added samples compared to those without extracts although the appearance scores were lower than those without extracts and body and texture was almost similar in all.

Physicochemical analysis

Change in pH

The pH of all the cheese samples was found to increase up to the 10th day of storage and thereafter it decreased till the end of storage in all the PCF samples without spice extracts Figure 2(a)A whereas the pH of the spice extract added PCF samples and control processed cheese increased till 20th day of storage and thereafter decreased till the end of storage Figure 2(b)A. However, no significant difference ($p > 0.05$) was found in pH among the samples during storage. Initial pH of 5.36 in control processed cheese increased to 5.49 on 20th day of storage and then decreased to 5.46 on 30th day of storage. Among the treated samples, PO showed the highest initial pH (5.55) followed by IN (5.45). The pH of IN decreased to 5.37 at 30th day of storage. Among spice extract added samples the initial pH of INF was lower than POF and PEA however, at 30th day of storage; the pH was similar in all the samples. The increase in the pH during the initial days of storage is non-significant ($p > 0.05$) however; the decrease in pH during storage could be due to the microbial spoilage. Reduction of pH during storage of processed cheese has been reported previously by Hussein et al. (2011) and Renuka et al. (2016).

Change in tyrosine content

Tyrosine content, which is a measure of degree of proteolysis significantly affects the acceptability of a product. All the stored samples were analyzed for tyrosine content with a view to monitor the proteolytic changes that occured during storage. The increase in tyrosine content in PCFs without spice extracts is shown in Figure 2(a)B and those with spice extracts in Figure 2(b)B. A gradual increase in tyrosine content of all the samples, irrespective of preservative treatment, was noticed during storage. At 0th day of storage no significant difference ($p > 0.05$) was found in the tyrosine content of the treated samples when compared to control. However, at 10th day of storage PO showed significantly higher ($p < 0.05$) tyrosine content of 1.62 mg/g compared to control (1.47 mg/g) and treated samples PE, (1.44 mg/g) and IN (1.37 mg/g). This could be due to the increased microflora in PO as a result of higher carbohydrate and moisture content favouring the growth of molds which might have caused more rapid proteolysis thereby increasing the amount of tyrosine as compared to control and other treated samples. At 20th day of storage a significant increase ($p < 0.05$) in the tyrosine value of PE (1.72 mg/g) was also observed compared to CPC which is again due to the increased microflora causing more rapid proteolysis. No significant change ($p > 0.05$) was observed in the tyrosine content of IN up to 20th day of storage however, it increased significantly to 1.76 mg/g at 30th day of storage.

The rate of increase of tyrosine content was slightly lower in spice extract added samples than those of without extract addition. Tyrosine content of 1.63 mg/g in POF at 10th day of storage increased to 1.79 mg/g at 20th day of storage compared to PO wherein it increased from 1.62 at 10th day of storage to 1.86 mg/g at 20th day of storage however no significant difference ($p > 0.05$) was found between 10th and 20th day of storage. Similarly PEA showed lower tyrosine content of 1.63 mg/g at 20th day of storage as compared to PE. INF also showed a lower tyrosine content of 1.61 mg/g at 30th day of storage as compared to 1.76 mg/g in IN. Spice extracts were found to slow down the rate of change of proteolysis in PCFs to some extent.

Changes in free fatty acid (FFA) content

Free fatty acid content of all the samples increased throughout the storage irrespective of the treatments as shown in Figure 2C. Initial FFA content of 3.04 in control processed cheese increased significantly ($p < 0.05$) to 4.07 at 30th day of storage however up to 20th day of storage the increase in FFA content was non significant ($p > 0.05$). At 20th day of storage a significant increase ($p < 0.05$) was observed in the FFA content of PO and PE compared to control Figure 2(a)C. However, no significant increase ($p > 0.05$) was found in the FFA content of IN. The significant increase ($p < 0.05$) in the FFA content of PO could be due to the increased activity of moulds in potato added cheese which had higher moisture and higher carbohydrate content whereas, in PE it could be due to the higher fat content. At 30th day of storage no significant increase ($p > 0.05$) was found in the FFA content of IN when compared to control.

Addition of spice extracts had a positive effect on the rate of change of lipolysis as shown in Figure 2C(b). It was found that POF and PEA did not show any significant increase ($p > 0.05$) in the FFA content compared to control however during storage however, a slight increase was observed. The rate of increase was lower in cheese samples with extracts compared to those without extracts. POF showed an FFA content of 3.25 at 20th day of storage which was lower than PO which showed an FFA content of 3.83 however, no significant difference ($p > 0.05$) was found between the two cheeses. Similarly PEA showed an FFA content of 3.28 which was lower than PE which showed FFA content of 4.05 at 20th day of storage. Similar trend was seen in INF.

Proteolysis - Alkaline Urea PAGE

The hydrolysis of proteins in processed cheese samples over a storage period of 30 days at 7-8°C was evaluated by alkaline urea-PAGE. Electrophoretrograms of all the cheese samples are shown in Figure 4- 6. Protein bands were identified by comparison with standards sodium caseinate where, β- Casein (β-CN) and α-

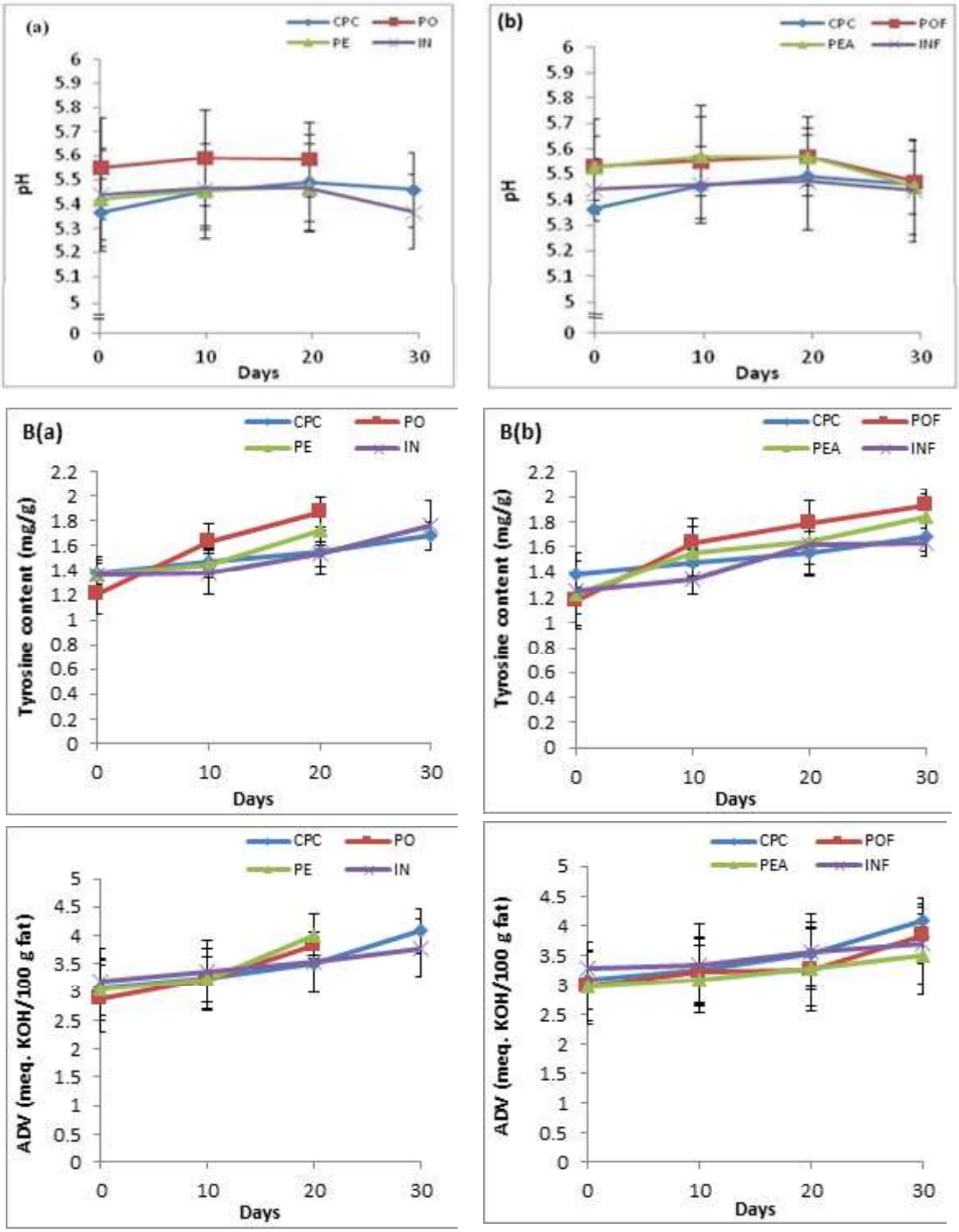


Fig. 2 Effect of non dairy ingredients on the: (A) pH, (B) Tyrosine and (C) FFA content of processed cheese during storage; (a) Without spice extracts, (b) With spice extracts; CPC- Control processed cheese, PO- Potato incorporated processed cheese, PE- Peanut incorporated processed cheese, IN- Inulin incorporated processed cheese, POF- Potato incorporated processed cheese with fennel extract, PEA- Peanut incorporated processed cheese with ajwain extract, INF- Inulin incorporated processed cheese with fennel extract.

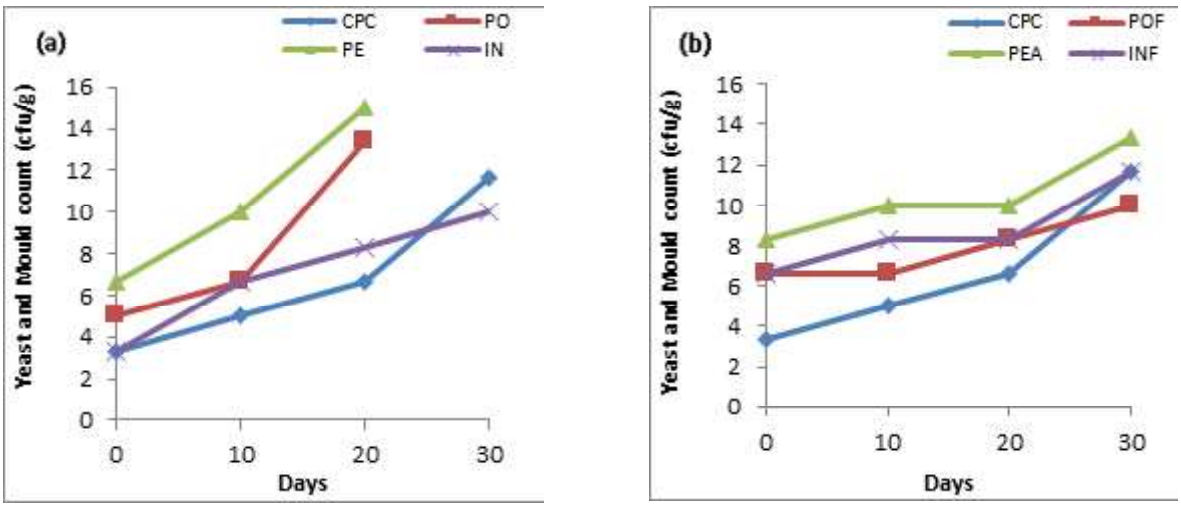


Fig. 3 Effect of non dairy ingredients on the yeast and mould count of processed cheese during storage; (a) Without spice extracts, (b) With spice extracts; CPC- Control processed cheese, PO- Potato incorporated processed cheese, PE- Peanut incorporated processed cheese, IN- Inulin incorporated processed cheese, POF- Potato incorporated processed cheese with fennel extract, PEA- Peanut incorporated processed cheese with ajwain extract, INF- Inulin incorporated processed cheese with fennel extract.

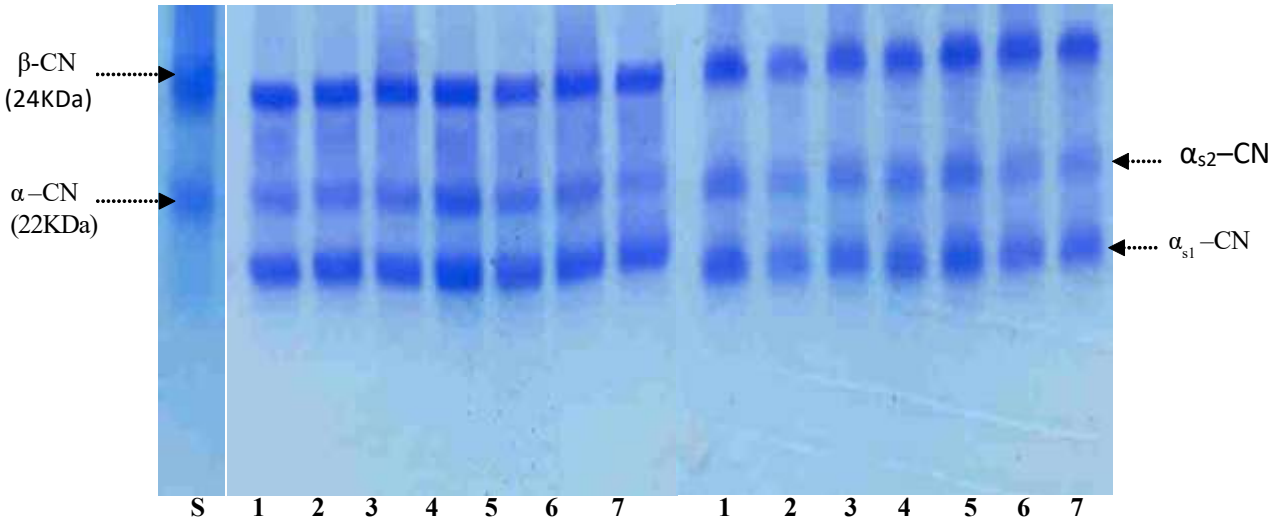


Fig. 4. Urea- Polyacrylamide gel electrophoretograms of processed cheeses: A -Zero day of storage, B-20th day of storage. S- Sodium caseinate standard, 1- Control processed cheese 2- Potato incorporated cheese, 3- Peanut incorporated cheese, 4- Inulin incorporated cheese, 5- Potato incorporated cheese with fennel extract, 6- Peanut incorporated cheese with ajwain extract, 7- Inulin incorporated cheese with fennel extra

Casein (α-CN) positions are shown (lane S, Figure 4). All the cheese samples show 3 prominent bands i.e., β- Casein, α_{s1}- Casein and α_{s2}- Casein. The cheese samples were made from ripened cheddar cheese where proteolysis of casein has taken place and show the visible bands of β- Casein, α_{s1}- Casein and α_{s2}- Casein. At 20th day of storage no further hydrolysis was observed in control cheese however a decrease in the intensity of α_{s2}- Casein> α_{s1}- Casein > β- Casein was observed in the treated samples and maximum decrease was observed in PO followed by PE (Figure 4B). The decrease in the intensity indicates further proteolysis which must be due to the higher rate of microbial growth in these samples. The higher carbohydrate and moisture content of the potato incorporated cheese might have favored the growth of microbes leading to more proteolysis. This was also confirmed from the tyrosine content of PO which showed higher tyrosine content compared to control and other cheese samples during storage Figure 2(a).

In contrast, POF and PEA showed lower degree of proteolysis. The electrophoretic patterns of cheese samples with and without

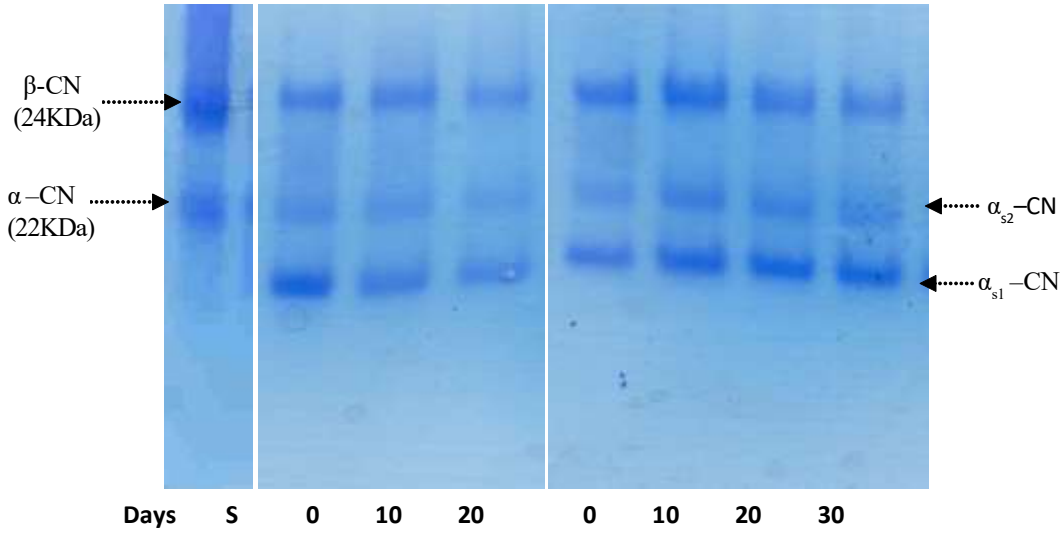


Fig. 5 Urea-Polyacrylamide gel electrophoretograms of Sodium caseinate standard (S), (A) Potato incorporated cheese, (B) Potato incorporated cheese with fennel extract.

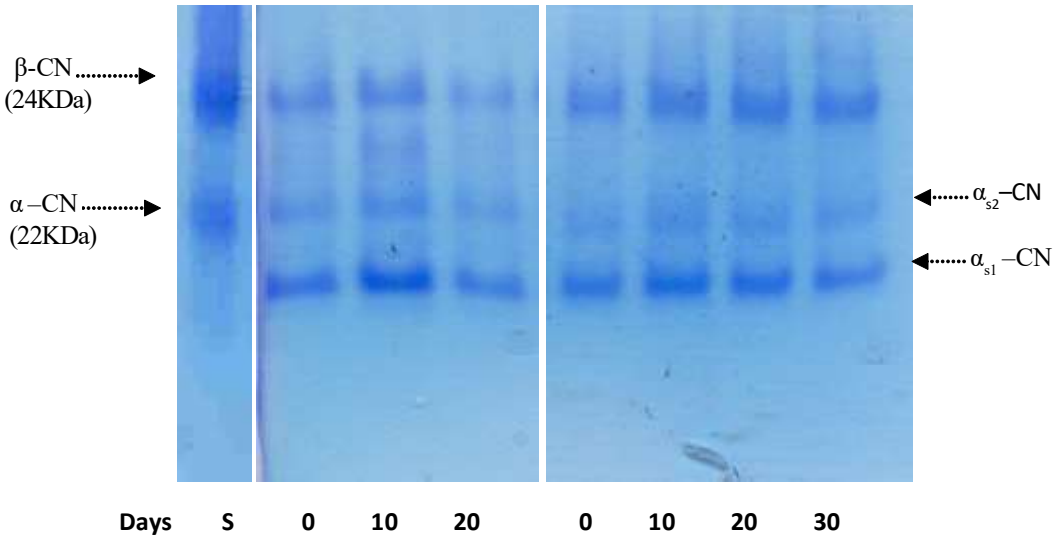


Fig. 6 Urea-Polyacrylamide gel electrophoretograms of Sodium caseinate standard (S), (A) Peanut incorporated cheese, (B) Peanut incorporated cheese with ajwain extract

spice extracts are shown in Figure 5-6. PO Figure 5(A) showed higher proteolysis than POF Figure 5(B) both at 10th and 20th day of storage. The α_{s2}-CN breakdown was more than α_{s1}-CN and β-CN as the intensity of α-CN became lower than β–CN. The higher proteolysis was due to the higher microbial load in PO. The addition of fennel extract to the potato incorporated cheese slowed down the degree of proteolysis to a greater extent as more visible bands are present in POF Figure 5b.

PE showed less proteolysis on 20th day of storage Figure 6(a) whereas, PEA did not show any proteolysis on 20th day of storage Figure 6(b). A slight degradation was observed in PEA on 30th day of storage. IN samples showed minimum proteolysis among all the PCFs and those containing fennel extracts did not show any proteolysis. Therefore, no electrophoretogram of inulin treated sample is shown.

Microbial changes

Changes in yeast and mould count

All the processed cheese samples were plated in Potato Dextrose Agar (PDA) to study the yeast and mould counts during storage. Yeast and mould count was found to increase during storage. At 0th day of storage yeast and mould count of 6.66 cfu/g was maximum in PE followed by 5.0 cfu/g in PO (Figure 3a). Control processed cheese showed initial yeast and mould count of 3.33

cfu/g. Yeast and mould counts increased as the storage progressed and it was found that yeast and mould count of PO and PE increased at a much higher rate as compared to control at 10th and 20th day of storage. Addition of spice extracts was found to slow down the rate of increase in yeast and mould counts. Initial yeast and mould count of 8.33 cfu/g in PEA increased to 10 cfu/g at 20th day of storage (Figure 3b). The rate of increase was lower than in PE wherein it increased from 6.66 to 15 cfu/g at 20th day of storage. Similar rate of change was observed in POA and INF.

The higher rate of increase in the yeast and mould counts in PE and PO might be due to the carbohydrate content of these cheeses however, lower rate of increase in extract added cheeses might be due to the antimicrobial properties of the extracts which might have suppressed the growth of yeast and moulds during storage thus improving their microbial quality. Similar results were found by Krumov et al. (2010) who reported that the addition of spice extracts (*Piper nigrum* and *Satureja hortensis*) improved microbiological quality of processed cheese significantly.

Changes in coliform count

In the present study, coliforms were found to be absent in all the fresh samples and no coliform growth was observed till the end of storage.

Conclusions

The addition of aqueous extracts of fennel and ajwain improved the microbial stability of processed cheese foods during storage. As a consequence, these spice extracts increased the shelf life of these products. Moreover, the flavour of non-dairy ingredient added cheeses is better maintained during storage contributing to improved overall consumer acceptability**.** These spice extracts can be considered to be used in the preservation of cheese. Further, the effect of essential oils from these two spices on the shelf life of cheese can be the next step in this research.

Conflict of interest

Authors declare no conflict of interest.

References

Abed KF (2007) Antimicrobial activity of essential oils of some medicinal plants from Saudi Arabia. Saudi J Biol Sci 14: 53-60

Awad S, Hassan AN, Halaweish F (2005) Application of exoploysacchride producing cultures in reduced cheese Cheddar cheese: Composition and proteolysis. J Dairy Sci 8: 4195-4203

Ceylan E, Fung DY (2004) Antimicrobial activity of spices 1. J Rapid Methods Autom Microbiol 12: 1-55

Creamer LK (1991) Electrophoresis in cheese. Bull IDF 261: 14-28

Cusato S, Gameiro AH, Corassin CH, Sant'Ana AS, Cruz AG, Faria JDAF, de Oliveira CAF (2013). Food safety systems in a small dairy factory: Implementation, major challenges, and assessment of systems' performances. Foodborne Pathog Dis 10: 6-12

Deeth HC, Fitzgerald CH (1976) Lipolysis in dairy products: a review. Aust J Dairy Technol 31: 53-64

Dias MAC, Sant'Ana, AS, Cruz AG, José de Assis FF, de Oliveira CAF, Bona E (2012) On the implementation of good manufacturing practices in a small processing unity of mozzarella cheese in Brazil. Food Control 24: 199-205

Guinee TP, Caric M, Kalab M (2004) Pasteurized processed cheese and substitute/imitation cheese products. In: Fox PF, editor. Cheese: chemistry, physics and microbiology. Volume 2: major cheese groups. 3rd ed. London, U.K.: Elsevier Applied Science 349–394

Gyawali R, Ibrahim SA (2012) Impact of plant derivatives on the growth of foodborne pathogens and the functionality of probiotics. Appl Microbiol Biotechnol 95: 29-45

Hayek SA, Gyawali R, Ibrahim SA (2013) Antimicrobial natural products. In A. Mendez-Vilas (Ed.), Microbial pathogens and strategies for combating them: Science, technology and education 2: 910-921

He W, Huang B (2011). A review of chemistry and bioactives of a medicinal spice: Foeniculum vulgare. J Med Plant Res 5: 3595-3600

Houghtby GA, Maturin LJ, Koenig EK (1993) Microbiological count methods. In: "Standard methods for the examination of dairy products".Chapter 6, Sixteenth Edn. (Ed. Marshall RT), Washington: American Public Health Association, pp. 213-246

Hussein FSE, Ibtisam M, El Zubeir M, Abdelaziz AF (2011) Quality Evaluation of Imported and Locally Produced Processed Cheese in Sudan. Jordan J Biol Sci 4: 231-236

Kapoor R, Metzger LE (2008) Process cheese: Scientific and technological aspects—A review. Compr Rev Food Sci Food Saf 7: 194-214

Meyer A (1973) Processed cheese manufacture. London, U.K.: Food Trade Press Ltd

Morsi NM (2000). Antimicrobial effect of crude extracts of Nigella sativa on multiple antibiotics-resistant bacteria. Acta Microbiologica Polonica 49: 63-74

Nagalakshmi S, Shakaracharya NB, Pura Naik J, Rao JML (2000) Studies on chemical and technological aspects of ajowan (*Trachyspermum ammi* (L.) Syn. *Carum copticum* Hiern) seeds. J Food Sci Technol 37: 277-281

Purohit P, Bohra A (2002) Antifungal activity of various spice plants against phytopathogenic fungi. Adv Plant Sci 15: 615-618

Rafiq SM, Ghosh BC (2017) Texture, meltability, color and sensory properties of processed cheese as affected by the addition of inulin. Indian J Dairy Sci 70:658-664

Rafiq SM, Ghosh BC (2017) Effect of peanut addition on the fatty acid profile and rheological properties of processed cheese. J Food Process Technol8: 690

Rafiq SM, Ghosh BC (2017). Effect of potato incorporation on the physico-chemical, textural and sensory properties of processed cheese. J Food Meas Charact 11: 776-780

Renuka V, Ramasamy D, Kumar DV (2016) Fortification of omega-3 fatty acids in processed cheese spread. Int J Sci Environ Technol 5: 2557-2565

Salek RN, Cerníkova M, Nagyova G Kuchar D, Bacova H, Minarcikova L, Bunka F (2015) The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. Int Dairy J 44: 37-43

Samples DR, Richter RL, Dill CW (1984) Measuring proteolysis in cheddar cheese slurries: comparison of hull and trinitrobenzene sulfonic acid procedures. J Dairy Sci 67: 60-63

Tajkarimi M, Ibrahim S, Cliver D (2010) Antimicrobial herb and spice compounds in food. Food Control 21: 1199-1218

Thakur N, Sareen N, Shama B, Jagota K (2013) Studies on in vitro antifungal activity of Foeniculum vulgare Mill. against spoilage fungi. Global J Bio Sci Biotechnol 2: 427-430

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RESEARCH ARTICLE

Development and characterisation of synbiotic whey beverage

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Abstract: Synbiotic foods has revolutionised the status of functional foods socially as well as commercially. The stringent environmental rules over disposal of whey and it's high nutritional value has driven industries to develop new whey based functional products. Among developed whey-based products fermented products has got significant position. In this study authors optimized a synbiotic whey beverage and also analysed for its proximate composition and storage stability. The developed synbiotic product had possessed following physico-chemical parameters: pH 3.67, acidity 0.90 per cent Lactic acid (LA), moisture 82.92 %, total solids 17.08 %, lactose 4.72 %, protein 0.43 % and fat 0.25 %. The beverage was found to have a probiotic count of 9.22 $\log_{10}$cfu/ml. Coliforms, yeast and molds counts were found to be absent for a period of 9 days. Rheological properties of whey beverage revealed that the product had a shear thinning behaviour with higher loss modulus (G'') values indicating viscous behaviour. Under refrigeration the drink started showing sign of spoilage from tenth day of storage. The required probiotic count was maintained in the product till 9th day of refrigerated storage as per legal requirement of FAO/ WHO (2002). From study it can be inferred that newly developed synbiotic whey drink has got shelf life of 9 days under refrigeration.

Keywords: Beverage, flow behaviour, Synbiotic, whey

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Introduction

Whey is a major by-product of cheese-making and casein manufacturing industry (Athira, et al. 2015). Whey is a green liquid obtained after coagulation of casein through action of chymosin (rennet) or mineral/organic acid. It has a yellow/green colour, or sometimes even a bluish tinge, but the colour depends on the quality and type of milk used (Mann et al. 2019). Nearly half of the total solids present in milk i.e. 6.4-7 % are transferred to whey. Whey from paneer was reported to have 6.4 % total solids, 0.5 %, 5.0 % lactose and 0.5 % mineral (Gupta and Singh, 2007). Apart from being a precious source of proteins, minerals and vitamins it is also a rich source of essential amino acids. The measure of amount of protein nitrogen that is retained by the body from a given amount of protein nitrogen that has been consumed is called 'Biological Value (BV)'. Whey proteins have high biological value (107) when compared to casein (77), egg (88) and soya proteins (59). The Protein Efficiency Ratio (PER) is used as a measure of growth expressed in terms of weight gain of an adult by consuming one gram of food protein. The protein efficiency ratio value of whey protein is three which is higher than wheat (1.00), rice (1.25), soya (2.12) and casein (2.50). The nutritive value of whey protein is next to that of egg proteins (Ganguly et al. 2019).

Despite of presence of valuable nutrients and functional bioactive peptides in whey, a large quantity is being unutilized owing its inferior sensory property. The drainage of whey into sewage also creates serious environmental problems owing to its high BOD content (30-60 g O_2/l) (Alvarez, et al. 2020). So a wide range of whey product developments like whey-protein concentrates, whey protein isolates (Liu et al. 2000), whey beverages and whey cheese are active consideration for complete utilization of nutrients present in whey. The whey beverages majorly includes fruit flavoured, fruit juice blended, carbonated, fermented, alcoholic whey beverages and nutrient enriched whey drinks (Kumar et al. 2013). In functional food market there is increased demand for the production of probiotic fermented beverages owing to their health beneficial effects and nutritional properties (Sabokbar and Khodaiyan 2015). However, in fermented whey beverages, probiotic viability during processing, storage and distribution remained a major challenge. Flavor of beverage

determines different desirable sensory characteristics. In order to increase consumer acceptability, the typical whey flavour drives to extraneous addition of flavor compounds. Citrus fruit and citrus fruit based flavors are being used to mask the unappealing taste of whey. In this context present study had been conducted to optimize synbiotic whey beverage and characterize the product in terms of its compositional, rheological and storage properties.

Materials and Methods

Collection and maintenance of culture

Lactobacillus casei (NCDC 298) was procured from National Collection of Dairy Cultures (NCDC), Karnal. Culture was activated in sterilized skim milk. Three consecutive transfers were done for maximum activation of culture. Routine maintenance of culture was carried out fortnightly in sterilized whey. In between the transfers, cultures were kept at-refrigerated temperature.

Preparation of synbiotic whey beverage

The buffalo milk whey was collected from University Dairy Plant, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy. It was filtered through muslin cloth and total solids content of whey was adjusted to 5.5 percent by adding pasteurised water. Inulin (Brenntag connecting chemistry company, India; 0.69% w/v of whey) and orange flavour (Sonarome, India) were added to the whey at 60°C followed by pasteurization at 72°C/15 sec. Pasteurized whey was inoculated with 1.5% *L. casei* at 40°C. Then it was incubated at 37°C for 16 h. Appropriate quantity of sugar was weighed and dissolved in pasteurized water (3:1 ratio) and then added aseptically to fermented whey at 37°C. The prepared product was packed in sterilized glass bottles and stored at refrigerated temperature.

For sugar optimisation, sugar was added at four different levels viz. 9% (TS1), 11% (TS2), 13% (TS3) and 15% (TS4) while flavour was added at a constant level of 0.03% w/v. For flavour optimisation Flavour was added at four different levels viz. 0.01% (T1), 0.02% (T2), 0.03% (T3) and 0.04% (T4) while sugar was added at a constant level of 11% w/v.

Sensory evaluation

Sensory evaluation of synbiotic whey beverage was carried out using 9- point Hedonic scale. The samples were drawn from the refrigerator immediately before serving to the panellists. Sensory evaluation panel consisted of five judges having adequate knowledge about the sensory evaluation methods and product characteristics were chosen from the faculty of College of Dairy Science and Technology, Thrissur. Sensory characteristics such as flavour, mouthfeel, appearance and overall acceptability were evaluated.

Physico-chemical analysis

pH of product was determined by using pH meter (Systronics, pH meter 361). The titratable acidity, protein, lactose and moisture of the sample was determined by the method recommended by AOAC (1990) for milk.

Color values

Color values (L*, a*, b*) of synbiotic whey beverage were measured using "Color-flex" colorimeter (MiniScan XE Plus, Hunter Associates Laboratory, Reston, Virginia, USA). Before the test, of the instrument was calibrated with standard black and white tiles as specified by the manufacturer. The output given by instrument was expressed in terms of CIELAB system.

Rheological analysis

The rheological measurement of whey beverage was performed by using dynamic rheometer (MCR 52, Anton paar, Ostifildern, Germany) fitted with cone and plate configuration (CP-75) of 75 mm diameter at 25°C. The product was loaded on rheometer base plate and allowed to rest for 10 minutes to maintain uniform temperature and to prevent the influence of structure modification during sample handling and loading. The flow curve of the symbiotic whey beverage was determined at 25°Cat variable shear rates ranging from 0 to $100s^{-1}$. The data was fitted to power law rheological model. For dynamic viscoelastic determination, rheological measurements were performed by frequency sweep test within the LVE range of the product. The rheological spectra was obtained recording dynamic complex viscosity (n*), storage modulus (G') and loss modulus (G'') as a function of frequency (1 to 100 Hz). Each test was carried out in triplicate.

Microbiological analysis

Probiotic count of each sample was estimated by pour plate technique, as described by Harrigan et al. (1989). Coliform, yeast and mold count of each sample was estimated by as per procedure outlined in IS: SP IX (1981).

Storage stability of synbiotic whey beverage

The optimized product was packed in glass bottles and kept at refrigerated temperature (5°C) for shelf-life study. The shelf-life of the synbiotic whey drink was assessed at three days intervals till spoilage. The changes in physico-chemical, microbiological and sensory parameters were assessed in the shelf -life study.

Statistical analysis

The results were analysed with SPSS v.16.0 for Windows software (SPSS South Asia (P) Limited, Bangalore, India). The data produced during optimization of sugar and flavour was analysed statistically using Krushkal-Wallis test followed by Mann-

Whitney u-test. The variation between different periods of measurements in the case of data related to probiotic count, pH, acidity, fat, lactose, moisture and total solids was analysed using repeated measures of ANOVA. In the case of storage study, sensory scores between measurements variability was analysed by using Friedman's test followed by Wicoxcan signed rank test.

Results and Discussion

Optimization of sugar level of synbiotic whey beverage

The data produced during sugar optimization was statistical analysed and results are presented in Table 1. Chi-square values were found to be significant ($p<0.01$) for all attributes indicating that there exist significant difference between treatments with respect to all attributes. Sugar percent has a significant effect on the mouthfeel, sweetness and total solids of synbiotic whey drink. Highest overall acceptability score (7.46) was obtained for the treatment TS2 which had a sugar content of 11% as compared to all other treatments. The relatively lower sensory scores was observed in of synbiotic whey beverage containing 15 % sugar. This may be due to the higher sweetness that resulted majorly from added sugar and to a little extent form inulin. Niness (1999) reported that inulin also contributes to sweet taste in foods nearly one tenth of sucrose sweetness. The panellists rated higher scores for TS2 treatment for overall acceptability and other sensory parameters. Hence sugar content was optimized to 11% in the synbiotic whey beverage. The amount of sugar optimized in various whey based beverages was reported to be in between 7-12% (Sthavarmath and Puranik 2018; Singh et al. 2014). Whey based low alcoholic (<1% alcohol) beverage called 'wheyvit' was developed by Bambha et al. (1972). It was reported to have a total sugar content of 10-11%. Optimised sugar content of this study much lower than sugar content in fermented whey beverage reported by Saha et al. (2017). However the perception of sweet taste varies with individual, age, gender and taste receptor genotypes (Mennella et al. 2018).

Optimization of flavour level of synbiotic whey beverage

Flavour was added to enhance the taste and improve sensory acceptability so as to mask the unpalatable salty taste of the final product. Data generated during flavor optimization was statistically analysed and results are presented in Table 2. Chi-square values was found to be Significant ($p<0.01$) for colour and appearance, mouthfeel, flavour and overall acceptability. Whereas sweetness values were found to be significant at 5% level of significance. Hence there exists a significant ($p<0.05$) difference between the treatments in respect to these attributes. No significant ($p>0.05$) difference was observed in terms of overall acceptability between T2 and T3. However, a mean score for overall acceptability was highest for the treatment T3 (8±0.05). Therefore, T3 was selected as optimised flavour percent.

Proximate composition of formulated synbiotic whey beverage

The final product was prepared by adding optimized level of sugar and flavor and its proximate composition was presented in Table 3. The moisture and total solids content of the synbiotic whey beverage developed in this study was found to be 82.92 and 17.08 per cent respectively. The protein and fat content of the product was 0.43 and 0.25% respectively per cent. The respective pH and titratable acidity of the freshly developed synbiotic whey beverage in this study was 3.67 and 0.9 per cent

Table 1 Effect of different levels of sugar on sensory attributes of synbiotic whey beverage

Parameter	TS1	TS2	TS3	TS4	Chi- square value
Color & appearance	6.44 ± 0.18^{b}	7.3 ± 0.17^{a}	6.9 ± 0.15^{b}	6.63 ± 0.19^{b}	9.425**
Mouthfeel	7.06 ± 0.17^{b}	7.33 ± 0.12^{a}	7 ± 0.1^{b}	6.3 ± 0.13^{b}	9.238**
Sweetness	6.87 ± 0.24^{b}	7.43 ± 0.3^{a}	7.03 ± 0.12^{b}	6.46 ± 0.17^{b}	9.877**
Flavour	7.13 ± 0.29^{b}	7.53 ± 0.06^{a}	7.27 ± 0.37^{b}	6.67 ± 0.06^{b}	8.897**
Overall acceptability	6.63 ± 0.18^{a}	7.46 ± 0.06^{a}	7 ± 0.1^{a}	6.47 ± 0.11^{a}	5.90**

Figures are the Mean± Standard error of three replications, **-significant at one percent level ($p<0.01$), $^{a-b}$ figures in row bearing different superscripts differ significantly

Table 2 Effect of different levels of flavour on sensory attributes of synbiotic whey beverage

Parameter	T1	T2	T3	T4	Chi- square value
Color & appearance	6.33 ± 0.18^{ab}	7.5 ± 0.15^{a}	8.12 ± 0.13^{a}	6.3 ± 0.52^{ac}	13.776**
Mouthfeel	6.17 ± 0.15^{ac}	7.47 ± 0.1^{a}	7.97 ± 0.08^{a}	6.3 ± 0.37^{bc}	14.02**
Sweetness	6.93 ± 0.22^{ac}	7.43 ± 0.24^{a}	7.97 ± 0.15^{a}	6.6 ± 022^{ab}	12.84*
Flavor	6.5 ± 0.27^{ab}	7.43 ± 0.14^{a}	8.1 ± 0.12^{a}	6.4 ± 0.34^{ac}	13.174**
Overall acceptability	6.57 ± 0.12^{ab}	7.34 ± 0.15^{a}	8 ± 0.05^{a}	5.97 ± 0.35^{cd}	14.02**

Figures are the Mean± Standard error of three replications, **-significant at one percent level ($p<0.01$), $^{a-b}$ figures in row bearing different superscripts differ significantly

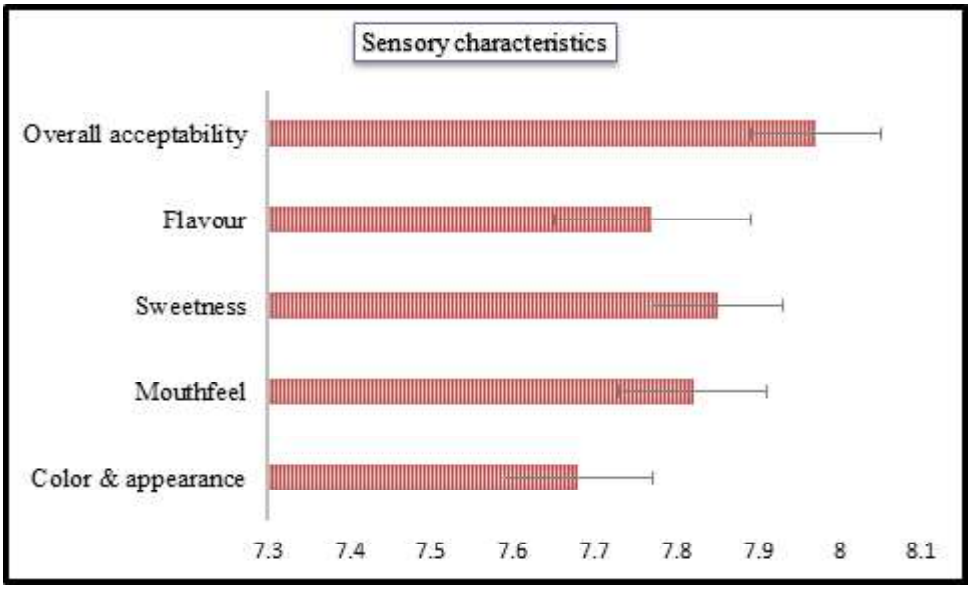

Fig. 1 Sensory characteristics of formulated fresh synbiotic whey beverage

Table 3 physico chemical, color and microbiological analysis of formulated synbiotic whey drink

Attribute	Observed values
Moisture (%)	82.92±0.33
Total solids (%)	17.08±0.33
Fat (%)	0.25±0.02
Protein (%)	0.43±0.02
Lactose (%)	4.72±0.03
pH	3.67±0.33
Titratable acidity (%LA)	0.90±0.34
Color values	
L*	20.78±0.09
a*	9.39±3.41
b*	25.18±2.73
Probiotic count (log_{10}cfu/ml)	9.22±0.012
Coliform count	Nil
Yeast and mould count	Nil

lactic acid (LA). The total lactose in the synbiotic whey beverage was 4.72 per cent which is slightly lower than the values reported for whey 5.2% (Gupta and Singh 2007). This decrease in lactose content can be attributed to fermentation of lactose to lactic by *Lactobacillus casei* NCDC 298. Conversion of lactose to lactic acid during fermentation of whey also reported by Vesa et al. (2000). The chemical composition of beverage is almost similar to that reported for various whey beverages (Sakhale et al. 2012; Singh et al. 2012). However the addition of inulin to the fermented whey beverage resulted slightly in total solids content than that of unfermented whey (data not shown). Similar trend of increase with addition of XOS also observed in strawberry-flavoured whey beverage (Souza et al. 2019). Color is an important characteristic of food from consumer's acceptability point of view. The L* value is a measurement of lightness and varies from 0 (black) to 100 (white); the a* value varies from -100 (green) to + 100 (red); and the b* value varies from -100 (blue) to +100 (yellow). Lightness (L*), Redness (a*) and Yellowness (b*) values of prepared beverage was ~ 20.78, 9.39 and 25.18 respectively. For neutral colors (white, gray or black) L*, a* and b* values approach zero, while with rise of these values, the color becomes more saturated or chromatic (Baccouche et al. 2013).

Microbial quality of synbiotic whey beverage

The viable *Lactobacillus* count of the synbiotic whey beverage was found to be 9.22 log_{10} cfu/ml (Table 3). "According to the guidelines stipulated by FAO/ WHO (2002) the probiotic strains should retain a viable count of at least 10^7 cfu/ml to exert the beneficial effect on the host". The results in this study indicate that synbiotic whey beverage fulfills specifications of FAO/WHO (2002). The absence of coliform count and yeast and mold suggest that adaption of hygienic practices during production ensured quality of the product. Moreover the high intrinsic acidity of synbiotic whey beverage suppressed the undesirable flora. The absence of coliforms and yeast and mold has been reported in earlier studies on probiotic whey based beverages also (Vandana et al. 2014).

Sensory characteristics of synbiotic whey beverage

The sensory scores of the synbiotic whey beverage (Fig. 1) in terms of colour and appearance, mouthfeel, sweetness, flavour and overall acceptability was found to be 7.68, 7.82, 7.85, 7.77 and 7.97 respectively on a 9 point hedonic scale. The developed synbiotic whey beverage has shown a overall acceptability score of 7.97 which is good indicative of the acceptable organoleptic quality for whey-based product.

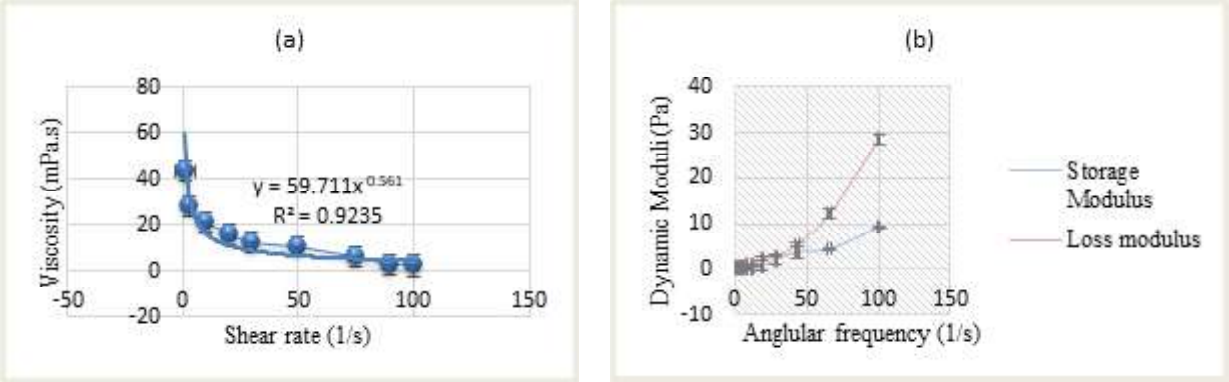

Fig. 2 (a) Flow curve (b) Dynamic Moduli of synbiotic whey beverage

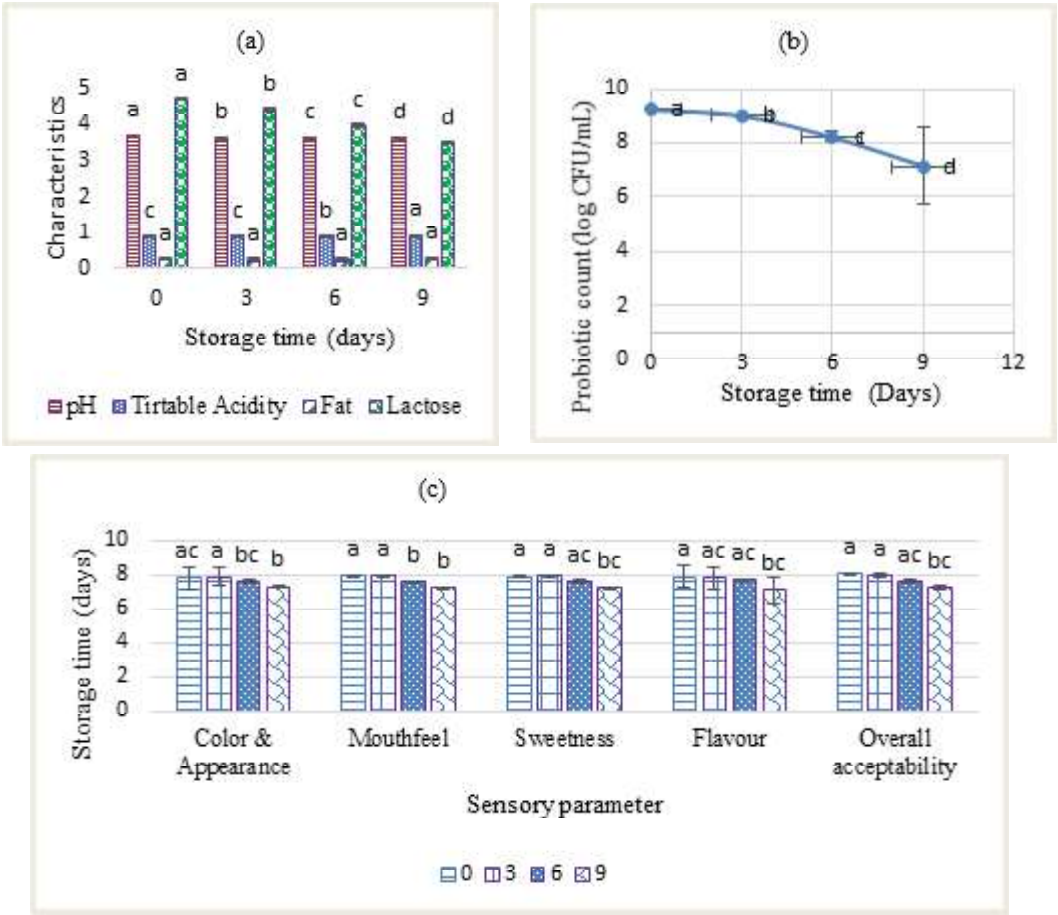

Fig. 3 (a) Changes in physico chemical (b) probiotic count (c) sensory characteristics of synbiotic whey beverage during storage period

Rheological properties of synbiotic whey beverage

Several rheological models have been studied to fit the viscosity data on lactic fermented whey beverages (Penna et al. 2003). The power law model has been used in modelling the flow behaviour of probiotic dairy beverages (Castro et al. 2013). The power law has expressed below

$$\sigma = K(\gamma)^{n}$$

here σ is the shear stress (Pa), k is the consistency index ($Pa \cdot s^{n}$), γ is the shear-rate (s^{-1}), and n is the flow index. The power law model was found suitable in this study to-express the shear stress data of the whey beverage at increasing shear rate. The consistency index (K), flow behaviour index (n) and correlation

coefficient (R^2) values for the model fit was 59.71, 0.561 and 0.826 respectively.

The viscosity of synbiotic whey beverage as a function of shear rate was graphically shown in Fig. 3 (a). The apparent viscosity of the synbiotic whey beverage was 10.6 mPa s at 25°C and 50 s^{-1} shear rate. Viscosity values of different fermented whey beverages made with different commercial probiotics were between 8 and 23.3 mPa s (Bulatovic et al. 2014; Oliveira et al. 2002). Generally, viscosity of the beverages decreased with increasing shear rate indicating a non-Newtonian behaviour (Masson et al. 2011). Synbiotic whey beverage has shown shear thinning behavior (n<1). Similar type of flow behaviour for prebiotic whey beverage also reported by Guimaraes et al. (2018).

The G' value is a measure of the deformation energy stored in the sample during the shear process, representing the elastic behavior of a sample. In contrast, the G'' value is a measure of the deformation energy used up in the sample during shear and lost to the sample afterward, representing the viscous behavior of a sample. As shown in Fig. 3 (b), loss modulus (G'') values were higher than the storage modulus (G') indicating viscous behaviour as for liquids. The observed results were in line with the results reported for fermented probiotic dairy beverage by Castro et al. (2013).

Storage stability of synbiotic whey beverage

The shelf-life assessment of the freshly prepared synbiotic whey beverage under refrigerated storage was done based on the changes in physico-chemical, microbiological, and sensory parameters at three days interval {Fig 3 (a), (b) & (c)}. From the results obtained during the storage period of 10 days, a decline in the pH was observed with values ranging from 3.66 to 3.62 with significant decrease ($p<0.05$). The titratable acidity slightly increased from 0.902 to 0.922 per cent Lactic acid (LA) was observed during the storage period from 0^{th} day to 9^{th} day. The titratable acidity of the synbiotic whey beverage increased significantly from three days to the end of the shelf-life. The similar results for probiotic drinks in terms of pH and titratable acidity values has been reported by various researchers (Vikram et al. 2011; Daneshi et al. 2012). The synbiotic whey beverage has shown a non-significant ($p>0.05$) changes in fat, moisture and total solids whereas lactose content changed significantly ($p<0.001$) from 4.708 % on 0^{th} day to 3.48 % on 10^{th} day of storage. The decrease in lactose content was due to the conversion of lactose present in whey to lactic acid because of fermentation. Hikmetoglu et al. (2020) also observed similar trends in lactose content of kefir i.e. from 2.8 g/100 g on first day to 0.3 g/100 g on 7^{th} day of storage.

The mean scores for changes in sensory attributes of synbiotic whey beverage during storage are presented in Fig 3 (c). The mean scores of all studied attributes during 10 days of storage are highest on 0^{th} day but lowest on 9^{th} day with significant difference ($p<0.01$). Sensory scores for overall acceptability of the synbiotic whey beverage decreased significantly ($p<0.01$) from 8.04 to 7.22. However there was no significant difference between 0^{th}, 3^{rd} and 6^{th} day.

The functional food claiming the status of synbiotic or probiotic product, minimum probiotic count that is necessary to exert their beneficial effect is 10^6 to 10^7cfu/ml (FSSAI, 2006). In the present study, viable *Lactobacillus* count of the synbiotic whey beverage decreased significantly ($p<0.05$) during the 10 days of storage. One log reduction in the viable count of synbiotic whey beverage was observed during 10 days of storage (Fig. 3 (b)). The possible reason for decrease in the *L. casei* NCDC 298 count was due to the physiological state of this strain. Similar trend in decrease of probiotic count also reported by Ismail et al. (2011) in whey based mango beverage. However the synbiotic whey beverage retained the recommended levels of live cells required to exert their beneficial effect up to 9 days of storage. The synbiotic whey beverage had shelf life of nine days under refrigerated storage. Vandana et al. (2014) also observed the 10 days keeping quality for fermented probiotic whey beverage. Heller (2001) reported that bacteria undergo more stress under storage conditions than cells in stationary phase during transition between the exceptional phase and the stationary phase. The pH values d"4.5 of probiotic foods can also effect the survivability of probiotic count (Gorski 1995). Our observation of storage stability of 9 days was in line with Saha et al. (2017), who also observed similar storage stability for channa whey fermented whey beverages.

Conclusions

Synbiotics are the synergistic combination of probiotics and prebiotics which helps in accomplishment of health benefits in host. Whey is a nutrient rich by-product of dairy industry which is not being utilized properly and disposed. Synbiotic whey beverage developed in this study is a good option for utilization of whey and valorisation of whey into a functional dairy product.

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References

Alvarez-Guzman CL, Cisneros-de la Cueva S, Balderas-Hernández VE, Smoliński A and De León-Rodríguez A. (2020) Biohydrogen production from cheese whey powder by Enterobacter asburiae: Effect of operating conditions on hydrogen yield and chemometric study of the fermentative metabolites. Energy Reports 6: 1170-1180

AOAC (1990) Official methods of analysis. (15th Ed.). Association Official Agriculture Chemists, Washington DC, USA

Athira S, Mann B, Saini P, Sharma R, Kumar R, Singh AK (2015) Production and characterisation of whey protein hydrolysate having antioxidant activity from cheese whey. J Sci Food Agric 95: 2908-2915

Baccouche A, Ennouri M, Felfoul I, Attia H. (2013) A physical stability study of whey-based prickly pear beverages. Food Hydrocolloids 33: 234-244

Bambha PP, Setty PAS, Nambudripad VKN (1972) "Whevit"—a nourishing soft drink. Indian Dairyman

Bulatovic ML, Krunic TZ, Vukasinovic-Sekulic MS, Zaric DB, Rakin MB (2014) Quality attributes of a fermented whey-based beverage enriched with milk and a probiotic strain. RSC Advances 4: 55503-55510

Castro WF, Cruz AG, Bisinotto MS, Guerreiro LMR, Faria JAF, Bolini HMA, Deliza R (2013) Development of probiotic dairy beverages: Rheological properties and application of mathematical models in sensory evaluation. J Dairy Sci 96:16-25

Daneshi M, Ehsani MR, Razavi SH, Labbafi M (2012) Effect of refrigerated storage on the probiotic survival and sensory properties of milk/ carrot juice mix drink. Electronic J Biotech 16: 0717- 3458

FAO/WHO [Food and Agriculture Organization/ World Health Organization] (2002) Guidelines for evaluation of probiotics in food*:* Report of a Joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. London, Ontario, Canada

FSSAI (2006) Food Safety and Standards Act (2006), Rules and Regulation (2011). (7th Ed.). New Delhi, India

Ganguly S, Sabikhi L, Singh AK (2019) Effect of whey-pearl millet-barley based probiotic beverage on Shigella-induced pathogenicity in murine model. J Funct Foods 54: 498-505

Gorski D (1995) International dairy foods: A culturally different world. Dairy Foods 96: 30-32

Guimaraes JT, Silva EK, Costa ALR, Cunha RL, Freitas MQ, Meireles MAA, Cruz AG (2018) Manufacturing a prebiotic whey beverage exploring the influence of degree of inulin polymerization. Food Hydrocolloids 77: 787-795

Gupta and Singh S (2007) Utilization of whey. Indian Dairy Association series No. 004/TE, IDA press, New Delhi.pp 4

Harrigan P, Zieman JC and Macko SA (1989) The base of nutritional support for the gray snapper (Lutjanus griseus): an evaluation based on a combined stomach content and stable isotope analysis. Bull Marine Sci 44: 65-77

Heller KJ (2001) Probiotic bacteria in fermented foods: product characteristics and starter organisms. The Am J Clinical Nutr 73: 374s-379s

Hikmetoglu M, Sogut E, Sogut O, Gokirmakli C and Guzel-Seydim ZB (2020) Changes in carbohydrate profile in kefir fermentation. Bioact Carbohydr Dietary Fibre 23:100220

Ismail AE, Abdelgader MO, Ali AA (2011) Microbial and chemical evaluation of whey-based mango beverage. Adv J Food Sci Technol 3: 250-253

Kumar S, Saxena D, Sabhiki L (2013) Developments in whey based beverages-A Review. Indian J Dairy Sci 66: 281-287

Liu G, Xiong YL, Butterfield DA (2000) Chemical, physical, and gel forming properties of oxidized myofibrils and whey and soy protein isolates. J Food Sci 65: 811-818.

Mann B, Athira S, Sharma, R, Kumar R, Sarkar P (2019) Bioactive peptides from whey proteins. In Whey Proteins (pp. 519-547). Academic Press

Masson LM, Rosenthal A, Calado VM, Deliza R, Tashima L (2011) Effect of ultra-high pressure homogenization on viscosity and shear stress of fermented dairy beverage. LWT-Food Sci Technol 44: 495-501

Mennella, J A, Nolden AA, Bobowski N (2018) Measuring sweet and bitter taste in children: Individual variation due to age and taste genetics. In Pediatric Food Preferences and Eating Behaviors (pp. 1-34). Academic Press

Niness KR (1999) Inulin and oligofructose: what are they? The J Nutr 129: 1402S-1406s

Oliveira MND, Sodini I, Remeuf R, Tissier JP, Corrieu G (2002) Manufacture of fermented lactic beverages containing probiotic cultures. J Food Sci 67: 2336-2341

Penna ALB, Oliveira MN, Tamime AY (2003) Influence of carrageenan and total solids content on the rheological properties of lactic beverage made with yogurt and whey. J Texture Stud 34: 95-113

Sabokbar N, Khodaiyan F (2015) Characterization of pomegranate juice and whey based novel beverage fermented by kefir grains. J Food Sci Technol 52: 3711-3718

Saha P, Ray PR, Ghatak PK, Bag SK, Hazra T (2017) Physico-chemical quality and storage stability of fermented Chhana whey beverages. Indian J Dairy Sci 70: 398-403

Sakhale BK, Pawar VN, Ranveer RC (2012) Studies on the development and storage of whey based RTS beverage from mango cv. Kesar. J Food Processing Technol 3: 1-4

Singh AK, Singh K (2012) Utilization of whey for the production of instant energy beverage by using response surface methodology. Adv J Food Sci Technol 4: 103-111

Singh S, Khemariya P, Rai A (2014) Process optimization for the manufacture of lemon based beverage from hydrolyzed whey. J Food Sci Technol 51: 691-699

Souza, FP, Balthazar, CF, Guimarães, JT, Pimentel TC, Esmerino EA, Freitas MQ, Cruz AG (2019) The addition of xyloligoosaccharide in strawberry-flavored whey beverage. LWT-Food Sci Technol 109: 118-122

Sthavarmath S, Puranik DB (2018) Development of pomegranate blended whey beverage. Int J Sci Env Technol 7: 1040-1046

Vandana, Shilpa V, Hati S (2014) Physico-chemical and sensory quality of probiotic fermented Whey drink and its storage study. Indian J Dairy Sci 67**:** 133-138

Vesa TH, Marteau P, Korpela R (2000) Lactose intolerance. J Am College Nutr 19: 165S-175S

Vikram Simha HV, Sharanakumar H, Udaykumar nidoni, Ramachandra CT, Tamil vendan K, Prakash KV (2012) Comparative studies on spray-drying and freeze-drying of pomegranate (*Punica granatum l.)* juice fermented with *L. acidophilus.* Int J Food Nutr Sci 1: 118-127

Indian J Dairy Sci 74(3): 215-223
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RESEARCH ARTICLE

Water vapour sorption thermodynamic properties and stability of spray dried avocado milkshake powder

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Abstract: Correlation of sorption and glass transition properties were determined for avocado milkshake pulp powder obtained by spray-drying with varying levels (2,6,12%) of maltodextrin (MD). The isotherms were Type III,and GAB model was found to be adequate to predict the sorption data. At 25 °C, the critical water content that ensures the glassy state of the shake powder during storage increased from 0.05 to 0.08 g water/g product while the critical water activity increased from 0.14 to nearly 0.6, yield increased, and stickiness decreased with increasing levels of MD. (2 to 12%). The correlation between glass transition temperature (Tg) and the water fraction present in the sample was fitted using the Gordon–Taylor model showing satisfactory values of R^2 (0.89). From the combined plot of Tg and equilibrium moisture content versus water activity, the critical water activity at 20° C is 0.1, which increased to 0.6 at -5°C.

Keywords: Avocado, Spray drying, Sorption, Glass transition temperature, Stability

Introduction

Among all the fruits, the avocado (*Perseaamericana*)is a fat-rich fruit mainly consumed fresh. Itis known for its pleasing taste and predominance of monounsaturated fatty acids (Duester, 2000). It is also recognized as a functional food containing health-promoting phytochemicals such as glutathione and beta-sitosterol (Rainey et al. 1994). Additionally, avocado is a good source of vitamins(A, B, C, E), minerals (potassium, phosphorus, magnesium, calcium, sodium, etc.), and fibre, offering significant health benefits. The preservation of avocado is a challenge to the food technologists due to browning and heat-induced sensory changes in the product. In order to increase commercialization on a large scale and give avocado an added value, it is essential to develop food products derived from this fruit with a shelf life long enough for their transportation and distribution to consumers (Dorantes et al. 2004).

Due to the growing market of dairy companies, there has been a merging of dairy products and fruit beverage markets, introducing hybrid dairy products that offer health, flavour, and convenience. Instant dairy mixes like milk powders, dry ice cream mix, and lassi powder do not contain phytonutrients. Very limited studies have reported the value addition and utilization of avocado in dairy products. There is a great scope for developing spray-dried dairy mixes fortified with phytonutrients derived from avocado pulp with suitable additives to reduce stickiness and heat-induced bitterness.

Drying produces a stable, easy-handling form that reconstitutes rapidly to a good quality product resembling the original one as close as possible. Nevertheless, drying of fruit juices and other high sugar content products with introduces practical complexities due to its thermoplasticity and hygroscopicity at high humidities and temperatures (Adhikari et al. 2004; Gabas et al. 2007). Consequently, the addition of maltodextrin (MD) and gums alongside other additives such as calcium silicate, carboxy-methyl cellulose, and pectin has been used in the production of powder juices (Bhandari et al. 2005). These characteristics are attributed to lower molecular weight sugars such as fructose, glucose, sucrose, and organic solids such as citric, malic, and tartaric which are major solids in fruit juices. The low glass transition temperature (T_g), high hygroscopicity, high water solubility and low melting point of these solids lead to a highly sticky product when spray-dried. The drying carriers or aides are compounds with high molecular weight that have high T_g; accordingly, they can raise the T_g value of feedstuff and the resultant powder (Shrestha et al. 2007). The hygroscopicity problems and thermo plasticity happening in drying of dairy-

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based or fruit juice with high sugar content can be overcome by adding some carriers such as MD and Arabic gum (Cano Chauca et al. 2005), waxy starch, etc. MDs have high water solubility and are mainly used in materials that are difficult to dry (Reineccius, 1991) and to reduce stickiness and agglomeration problems during storage, thereby improving product stability (Silva et al. 2006). Sugar and tricalcium phosphate were found to enhance the flowability of powders with high-fat content (Shivakumar et al. 2012). The fat in the cream powder was encapsulated by lactose or maltodextrin, and casein resulted in cream powder with good emulsion properties and solubility as those of the cow milk powder(Xiong et al.2004).Moisture sorption isotherms illustrate the relationship between water activity and equilibrium moisture content of a food product. Familiarity of water sorption isotherms and isosteric heat of sorption is of essential importance to numerous food processes such as storage, drying, and packaging since they are used to calculate drying time, to predict ingredients behaviour upon mixing, packaging selection, modelling moisture changes that occur during storage, and estimating shelf-life stability, which is very important mainly for food powders. These properties give information about the interactions between food components and the water sorption mechanism. They also help to establish the final moisture content and permit the estimation of energy requirements of the drying process.

Numerous mathematical models for the description of foods moisture sorption behaviour are available. Some of these models are based on theories on the sorption mechanism; others are purely empirical or semi-empirical. The principles used to select the most appropriate sorption model are the degree of fitting of experimental data and the physical meaning of the model. Recently, the concepts related to water activity have been coupled with those of the glass transition temperature, Tg, providing an integrated approach to the role of water in foods. The glass transition is defined as the temperature at which an amorphous system changes from the glassy state to the rubbery state. Molecular mobility in the glassy state is extremely slow due to the high viscosity of the matrix (about 10^{12} Pa s). Therefore, the Tg can be taken as a reference parameter to characterize the properties, quality, stability and safety of food systems. Structural alterations, such as stickiness, agglomeration, caking and crystallization, occur in amorphous food powders when stored at temperatures above the Tg. Foods with low-moisture contents and Tg value above the storage temperature can be considered stable. However, a slight increase in moisture significantly reduces the Tg. Therefore, the moisture sorption and Tg supply critical values for the water activity and moisture content at room temperature (Khalloufi et al. 2000; Roos1995; Roos and Karel, 1991).

The aim of the present work was to evaluate the influence of varying levels of maltodextrin on the physical properties, water sorption, Tg, and stability of spray-dried avocado milkshake powder. Modeling of the sorption isotherms and Tg using selected models from the literature was studied, and attempts were made to couple sorption data with Tg for better prediction of the stability of the product.

Materials and Methods

Materials

Avocados were purchased from a local market (Mysore, India). The avocados were stored in a cold chamber at 4°C and thawed according to the quantity required to produce the spray-dried milkshake powder. Milk was procured from Nandini Dairy (Mysore, India). The carrier agent used was Maltodextrin (DE 10), procured from Pristine Organics, Bangalore.

Preparation of avocado milkshake

Avocados (*Persea americana Mill*.) were washed, disinfected with Potassium permanganate. Then the pulp was separated from the peel using a sterilized knife. The extracted pulp was ground in a mixer-grinder. The fine ground pulp was homogenized with milk along with powdered sugar, and carrier material maltodextrin (DE 10) was added at the rate of 2, 6, and 12% of the blend containing milk and avocado pulp. Sugar was added at the rate of 10%.

Spray drying

Milkshake was prepared by mixing milk and pulp in the ratio 3:1 with the addition of 10% sugar. Maltodextrin (DE 10) was added at 2, 6 and 12% of the milk shake blend without sugar. The spray drying process was performed using a laboratory spray dryer (S.M Scientific, Kolkata). The dryer was equipped with a spray nozzle with an orifice of 0.7 mm in diameter. The avocado milkshake was fed into the drying chamber using a peristaltic pump. The inlet air temperature was 175ºC, and the outlet air temperature varied from 90 to 100ºC for each sample. The feed pump rpm was 20, feed rate was 500 ml per hour and the blower speed was 2350 rpm during the spray drying process. The compressed air pressure was maintained between 2-3 kg/cm^2 corresponding to air flow rate of 0.66 – 1.33 CFM.

Proximate analysis

The samples with varying levels of MD were taken and analyzed for moisture, protein, and fat according to the AOAC, 2005 methods. Moisture and protein determinations were performed in triplicates, and single fat analysis was conducted for each sample. The powder sample (5-10g) was incinerated in a muffle furnace at 550ºC for 3-4 hours to determine the ash content.

Modelling of sorption isotherms

Sorption isotherms were determined by means of the gravimetric method. The initial moisture content in powder samples were

determined by drying in a vacuum oven (Ranganna, 2004). Two to three grams samples of powder filled in sterilized glass bottle weighing dishes were placed in six separate desiccators containing saturated salt solutions for maintaining relative humidity (RH) levels from 11 to 85 %. The six jars were placed in an oven adjusted to a stable temperature for 24 h in order to bring the salt solutions to a constant temperature. Triplicate samples were used (2–3 g each) equilibrated over saturated salt solutions (l, providing relative humidity values of 11.15%, 32.73%, 43.80%, 52.86%, 75.32% and 84.32%, respectively in desiccators at 25°C until equilibrium. The air inside the desiccators was removed with the help of a vacuum pump. A glass dish containing 5 ml toluene was placed in desiccators with relative humidity higher than 75 % to check mold growth. The samples were weighed periodically till they attained equilibrium, after which they were analyzed for moisture content. To establish moisture sorption isotherms, the equilibrium moisture contents, determined by static gravimetric method, were plotted against water activity. The hygroscopic equilibrium of samples was reached in 7-10 days. The equilibration moisture content in samples were determined by subtraction method and expressed as g water/100 g solids. To establish moisture sorption isotherms, the equilibrium moisture contents were plotted against water activity. The physical appearance of the samples was also observed to check whether the powder had suffered any transformation such as agglomeration, caking or collapse.

Several models (empirical, semi-empirical, and theoretical) with two or more parameters have been used in the literature to describe the sorption isotherms. Equations based on sorption theories, such as BET and GAB models, are usually preferred by most researchers, since some physical meaning may be attached to their parameters, aiding in the understanding of the water sorption phenomena. Derived by simple extension and generalization of Langmuir's theory of unimolecular adsorption, the classic BET Eq. (1) (Brunauer et al. 1938) is a two-parameter model assuming the condensation of an infinite number n of layers from the vapor phase onto the adsorbent surface.

$$Xe = \frac{Xm\ C\ \ aw}{[(1-aw)(1-aw+C\ aw)]} \quad \text{Eq. (1)}$$

Xe: equilibrium moisture content (g water/g dry matter), Xm: monolayer moisture content (g water/g dry matter),a_w: water activity, C: constant of BET.

GAB model shown in Eq 2 was also used to fit experimental data.

$$Xe = \frac{Xm\ C\ K\ aw}{[(1-K\ aw)(1-K\ aw+C\ K\ aw)]} \quad \text{Eq. (2)}$$

Xe: equilibrium moisture content (g water/g dry matter), Xm: monolayer moisture content (g water/g dry matter),a_w: water activity, K and C: constant of GAB and BET, respectively.

In order to obtain the model parameters, a non-linear regression analysis was carried out using the Graphpad Prism (USA) software package. The degree of fitness of each model was evaluated by the determination coefficient and mean relative deviation modulus E

$$E = \frac{100}{N}\sum_{i=1}^{N} \frac{|Ve-Vp|}{Ve} \quad \text{Eq. (3)}$$

N: population of experimental data, Ve and Vp: experimental and predicted value, respectively.

Glass transition temperature

About 10 mg of avocado milkshake powder were placed into differential scanning calorimetry (DSC) aluminum pans, which was equilibrated over saturated salt solutions in desiccators at 25ºC until equilibrium was reached. The samples were then hermetically sealed with lids for analysis and weighed. The mass of each sample pan was matched in advance with the mass of an empty reference pan to within ± 0.1 mg. The DSC analyses were carried out in a TA-MDSC-2920 (Ta Instruments, New Castle, De, USA). For temperatures below 70ºC, liquid nitrogen was used. After cooling the sample to -30ºC, the glass transition temperature was determined on thermo-analytical curves obtained by heating the sample at 10ºC/min up to 110ºC (or other values for the initial and final temperatures, according to the sample). The second scanning of each sample was performed to reduce the enthalpy relation of the amorphous powder, which appears in the first scan. All analyses were done in triplicate and the data were treated by the software Universal Analysis 2.6 (TA Instruments, New Castle, De, USA).

To describe the plasticizing effect of water on avocado milkshake powder, the glass transition temperature data were fitted to the Gordon-Taylor model (Gordon and Taylor, 1952)

$$Tg = \frac{WsTgs+kWwTgw}{Ws+kWw} \quad \text{Eq. (4)}$$

W: weight fractions (g/g total), s: solids, w: water, Tg: glass transition temperature (°C), k: constant.

The Tg_w value was taken at 135°C (Johari et al. 1987).A non-linear regression analysis was carried out using the Graph Pad

Prism (Ohio, USA) software package to obtain the model parameters k and Tgs.

Results and Discussion

Proximate composition and quality characteristics of spray-dried avocado milkshake

Fresh avocado showed moisture content in the range of 65-75%, total acidity of 1.02± 0.39%(as citric acid content), and pH 6.38± 0.02, respectively. The fresh pulp had a total solid content of 8 - 9 ºBrix. The milk used in formulation of butter fruit milkshake (BFMS) samples contained 3% fat and 8.5% SNF. The proximate composition of BFMS before drying are shown in Table 1.

Table 1 Proximate Composition and Quality characteristics of butter fruit milk shake (BFMS)

Quality Parameter	BFMS*
Moisture content (%)	79.6± 0.5
Ash (%)	3±0.04
Protein (%)	2.4±0.02
Fat	6± 0.1
Carbohydrate (%)	9±0.2
Acidity (% citric acid)	1.03±0.03
pH	6.3±0.1
TSS(Brix)	17± 1

*Values shown are mean ± standard deviation of 6 samples

Spray dried avocado milkshake powder

Spray dried avocado milk shake (BFMS-SD) powder containing varying level of MDs were evaluated for colour, water activity, sorption behaviour and glass transition temperature. BET and GAB models were used to fit the experimental sorption data, and the monolayer moisture content in spray-dried samples were estimated as parameters of these models. DSC was used as a tool to evaluate the T_g and endothermic and exothermic phase transitions in the products over a range of -30 to 110 °C.

Colour values and water activity

L*, a*, b* values and water activity of fresh and spray dried avocado milkshake containing varying levels of maltodextrin (MD) were estimated using Hunter lab colourimeter, and the results are shown in Table 2. Color values of BFMS-SD powders differed significantly from the colour values of fresh avocado pulp (data not shown). With increasing level of MD there was slight variation in values, but there was no specific trend observed. The BFMS-SD containing 12 % MD showed the highest L value indicating maximum deviation from the greenish colour of pulp due to addition of various additives and treatment given. The addition of varying levels of MD did not affect a_w values of BFMS samples. The powder samples obtained after drying differed significantly ($P<0.05$) in a_w values based on the content of MD present. The a_w of BFMS-SD varied between 0.205 to 0.311 depending on the content of MD. BFMS powder is stable with respect to lipid oxidation, non-enzymatic browning, enzyme activity, and of course, the various microbial parameters with water activity in the range mentioned. As a_w increases, the probability of the food product deterioration increases (Rahman, 2007). The stability of food with respect to oxidative changes is maximum at 0.4 and is in the a_w range 0.2 to 0.6 Water activity less than 0.2 or higher than 0.6 has an adverse effect on the oxidative stability of the product (Rahman, 2010). As avocado pulp contains high-fat content compared to other fruit pulps, the oxidative stability of the product is of great significance. Water activity is a crucial aspect that determines shelf life. The maltodextrin added powder was found to have good stability than plain BFMS powder. It was observed that when the level of maltodextrin was increased, the water activity reduced; hence the product become more shelf-stable.

Sorption isotherms

The correlation between water activity (a_w)and water content is complex. An increase in a_w is usually be associated with by arise in water content, however in a non-linear pattern. This correlation between a_w and moisture content at a given temperature is called the moisture sorption isotherm. These curves are determined experimentally and constitute the fingerprint of a food system. Isotherms can be employed to help predict product stability over time in different storage conditions. The knowledge and understanding of sorption isotherms are extremely crucial in food processing for the design and optimization of drying equipment, design of packages, predictions of quality, stability, shelf-life, and for calculating moisture changes that may occur during storage. Equilibrium moisture content for avocado milkshake powders added with varying levels of maltodextrin were plotted against at the six water activities. The sorption isotherms showed an increase in equilibrium moisture content with increasing water

Table2 Color and water activity values of spray dried avocado milkshake (BFMS)powder containing varying levels of maltodextrin (MD)

Avocado milk shake Powder	L*	a*	b*	Water activity
BFMS-SD 2% MD	85.46± 0.2	-4.51± 0.01	21.41±0.1	0.311±0.05
BFMS-SD 6% MD	85.5±0.1	-2.59±0.02	17.55±0.1	0.27±0.01
BFMS-SD 12% MD	87.24±0.2	-2.81±0.01	15.84±0.05	0.205±0.02

*Values shown are mean ± standard deviation of 3 samples

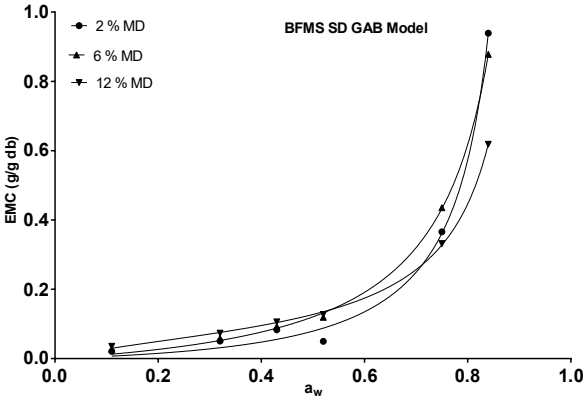

Fig 1. Water sorption isotherms (GAB Model) of spray dried avocado milkshake powder (BFMS-SD), formulated with 2, 6 and 12% maltodextrin (MD)

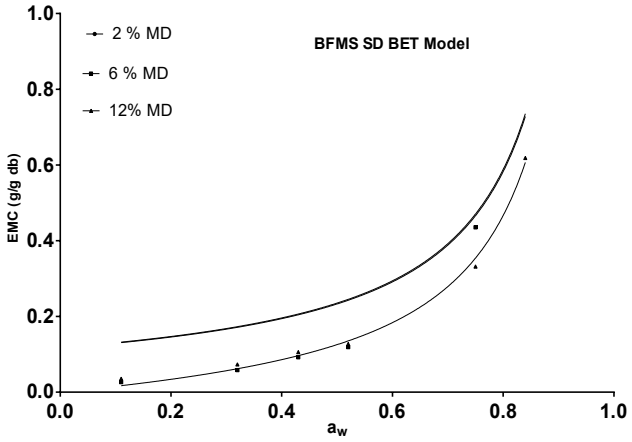

Fig 2. Water sorption isotherms (BET Model) of spray dried avocado milkshake powder (BFMS-SD), formulated with 2, 6 and 12% maltodextrin (MD)

Table 3 Estimated parameters of GAB and BET models fitted to sorption data of spray dried avocado milkshake (BFMS)powder produced with different levels of maltodextrin (MD)

Avocado milk shake	GAB				BET		
Powder	Xm	C	k	R^2	Xm	C	R^2
BFMS-SD 2% MD	0.0970	0.4992	1.0870	0.9957	0.1165	-9.452	0.7944
BFMS-SD 6% MD	0.1311	0.7219	1.0420	0.9992	0.1177	-2.473	0.8653
BFMS-SD 12% MD	0.0726	4.3410	1.0550	0.9995	0.1114	1.293	0.9949

activity, at constant temperature. These isotherms can be classified as type III, according to Brunauer's classification, characteristic of non-porous or macroporous solids due to weak gas-solid interactions. The weakness causes uptake at lower water activity to be small. But once a water molecule has become adsorbed, the adsorbate- adsorbate forces will promote the adsorption of further water molecules and the resulting isotherms will become convex to pressure axis.The sorption isotherms fitted to GAB and BET models are presented in Figure 1 and 2. These curves are used to estimate the coefficients of two sorption models.

At a given water activity, EMC of the samples decreased with increasing levels of MD. Similar results were reported by other workers (Gabas et al. 2007; Mara and Maria, 2005). The presence of additives in the avocado milkshake powder probably modified the balance of hydrophilic/hydrophobic sites, promoting a decreased amount of sorbed water as reported by Perez-Alonso et al. (2006) for pure and blended carbohydrate polymers.

Similar isotherms were observed for protein hydrolysates from fish, pineapple, tomato pulp, West Indian cherry and lactose hydrolysed skim milk powders (Gabas et al. 2007; Shrestha et al. 2007). This type of curve was also observed by, Gabas et al. (2007) for vacuum dried pineapple containing maltodextrin and gum Arabic, and Kurozawa et al. (2009) for spray-dried chicken meat hydrolysate protein produced with these same carrier agents.

Experimental data of the sorption isotherms for spray-dried avocado milkshake powder produced with different levels of carrier agent maltodextrin was fitted to GAB and BET models. Each model was tested for adequacy and goodness of fit by determining the coefficient R^2. The estimated parameters with the coefficient of determination (R^2) are presented in Table 3.

Both BET and GAB models showed a good fit to experimental data, with high R^2. The results showed that for all the different MD concentrations studied, the GAB model presented a better fit than the BET model, with coefficient of determination close to unity. Hence GAB model gives a better prediction of the adsorption behavior of both SD avocado milk shake powder. As the R^2 values calculated by the BET model were lower, this model lacked prediction accuracy than GAB model for the sorption data of powdered avocado milkshake. This can be explained due to the limiting values for the constants C_{BET} as suggested by Lewicki (1997), established on the mathematical analysis of the model. For sigmoidal type curves, constants values are in the range $0.24 \ll K_{GAB}$ 1 and 5.6 C_{GAB}, to guarantee a relatively good description of the isotherms and to fulfill the requirements of the GAB model, as well as assuring that the calculated monolayer

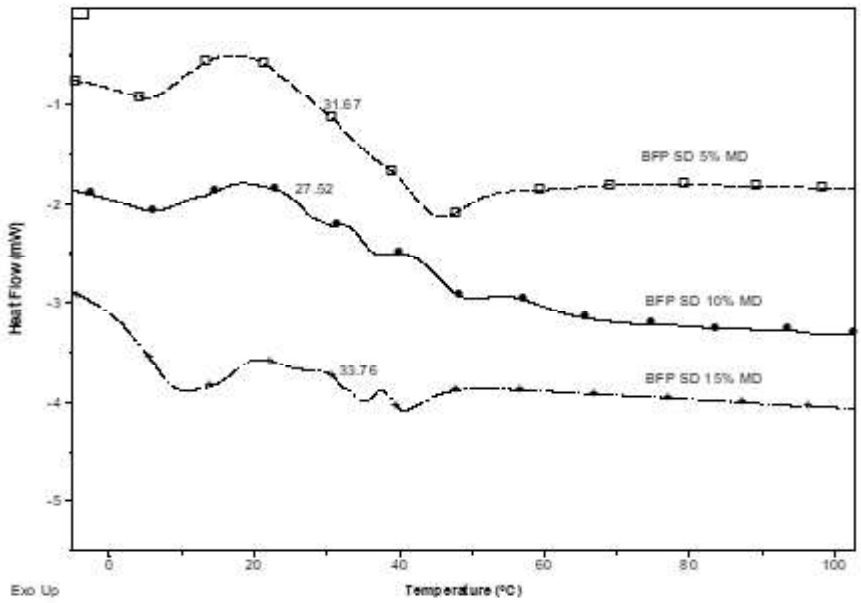

Fig 3. Thermograms of spray dried avocado milkshake powder (BFMS-SD), formulated with 2, 6 and 12% maltodextrin (MD)

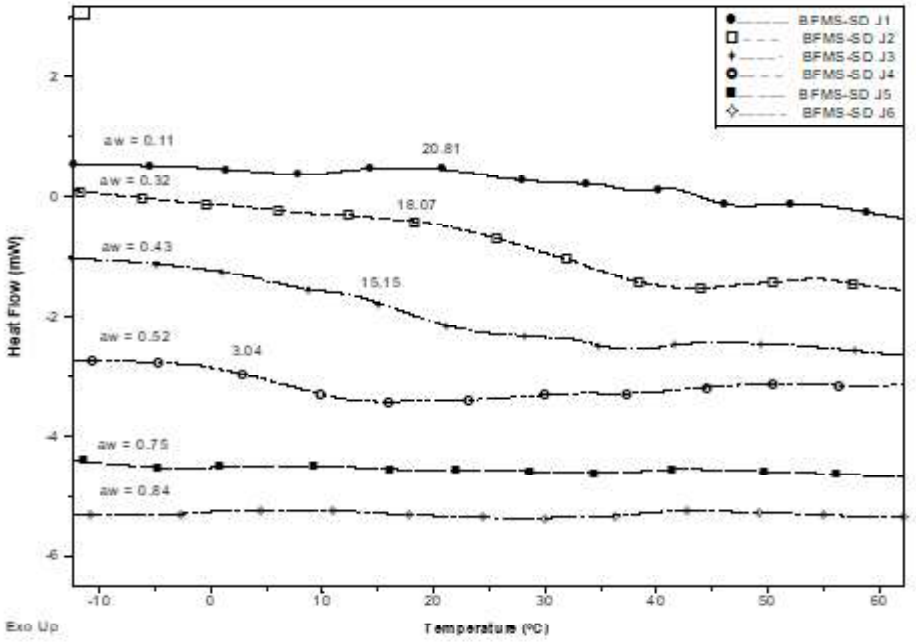

Fig. 4 Thermograms of spray dried avocado milkshake powder (BFMS-SD) equilibrated at different water activities and formulated with12% maltodextrin

moisture content values differed by no more than 15.5% from the true monolayer capacity.

For samples containing 12% MD, both the models showed good degree of fit to predict the sorption behavior. Both BET and GAB models are based on the monolayer moisture concept and give the value of monolayer moisture content of the material (Xm), considered as the safe moisture for dried foods during preservation, while most other models lack this parameter. The monolayer moisture content (Xm) indicates the amount of water that is strongly adsorbed to specific sites at the food surface and is considered an important value to assure food stability. Xm values were obtained from the linear plot of a_w versus 1/(EMC*(a_w-1)) and calculated using the equation Xm=1/(s+i) where s is the slope and i is the y-intercept. TheXm values obtained by fitting BET & GAB models to sorption data of BFMS-SD containing varying levels of MD varied from 0.07 to 0.13 g/g db.

DSC thermograms of BFMS powder

BFMS-SD samples containing varying levels of MD were analysed in the temperature range -30°C to 110°C at the rate of 10

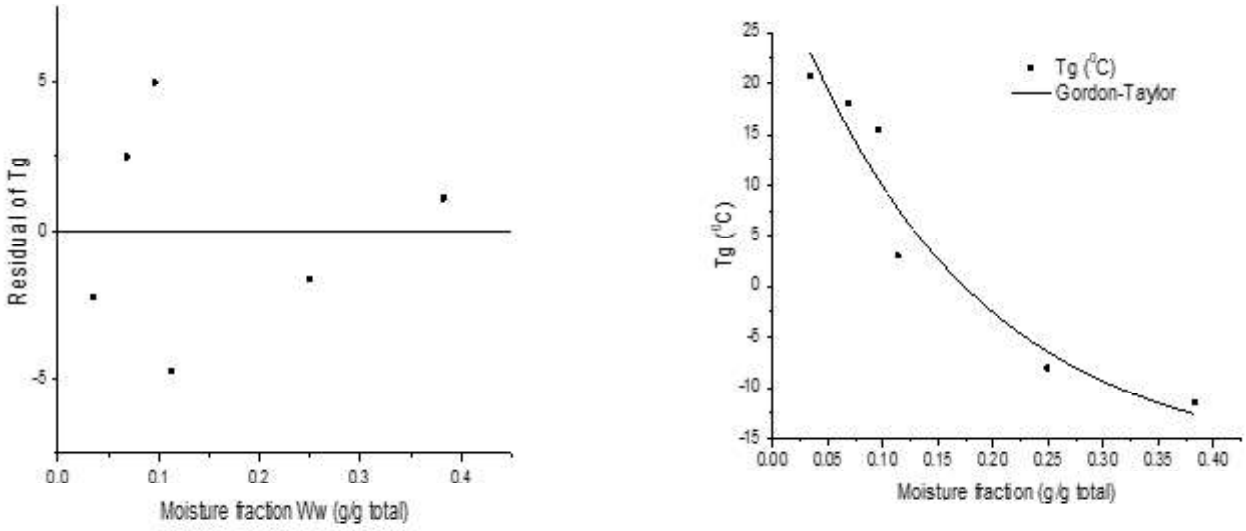

Fig. 5 Residual plots obtained for spray dried avocado milkshake powder (BFMS-SD) and Gordon –Taylor fitting for moisture vsTg values containing 12 % maltodextrin (MD)

°C /min and the results obtained are summarized in this section. The thermograms of spray-dried BFMS powders containing 2, 6 and 12% MD had Tg values 26.1, 27 .52, and 33.9 respectively showing increase with increasing levels of MD in the blend. MD upto 12% was required for spray dried samples to avoid stickiness during drying and also to increase the product yield. A satisfactory product cannot be obtained with 2% MD with spray drying technique.

DSC thermograms of BFMS samples equilibrated at different RH conditions

DSC thermograms of selected BFMS samples (SD sample containing 12% MD) were analyzed to study the plasticizing effect of water. In general, the thermograms showed the typical second-order transition that produces a step change in the heat flow due to changes in heat capacity at the temperature of phase transition. The glass transition temperature was taken as the midpoint of the glass transition. Glass transition temperature of powders obtained with different drying methods equilibrated at different RH conditions varied from -8 to 20.81° C. The changes in exothermic and endothermic phase transitions due to changing moisture content in BFMS-SD samples were analysed.

Tg values for samples equilibrated at different water activities were correlated with sorption data (Figure 4).Tg decreased with increasing water activity. Plasticisation by an increasing water content results in the decrease of the glass transition. At the critical water activity, the Tg is decreased to storage temperature and further increases in a_w result in a decrease in viscosity of particles, stickiness, caking, and rapid increases in rates of lactose crystallisation and diffusion-controlled reactions. Two endotherms and one exotherm were observed in most of the thermograms analysed for plasticizing effect of water. At higher water activities the onset of crystallization differed significantly whereas the changes in melting curves were not significant due to the plasticizing effect of water. The crystallization peaks and melting peaks shifted to lower temperatures with increasing moisture content indicating the lower stability of samples containing high moisture. These differences were highly significant in samples equilibrated at a_w of 0.75 and above.

The correlation between Tg and the water fraction present in the sample was fitted using the Gordon–Taylor model (Figure 5) showing satisfactory values of R^2 (> 0.85). The sticky-point temperature is normally about 10-23ºC higher than the glass transition temperature and, in spray drying, particles which are above this temperature stick to the dryer wall and degrade, and/ or clump together, adversely affecting the free-flowing property. In the case of avocado milkshake powder, considering its low sugars and acids level, the sticky-temperature is much higher than 85ºC (the outlet air temperature) and that would result in a high degree of stickiness and thus help in a significant powder yield. There spray drying could be efficiently employed for the production of avocado milkshake powder. In a study on skim milk powder, it is reported that the glass transition temperature was found to be virtually the same as the sticky-point temperature measured using a thermo-mechanical test.It has been shown in previous reports that Ts and Tg (glass transition temperature) are very closely correlated, and both can be used to assess stickiness of powder materials (Ozmen and Langrish , 2002; 2006). As Tg is dependent on sugar and acid composition, stickty point (Ts) also varies based on these.

Product stability based on water activity and glass transition

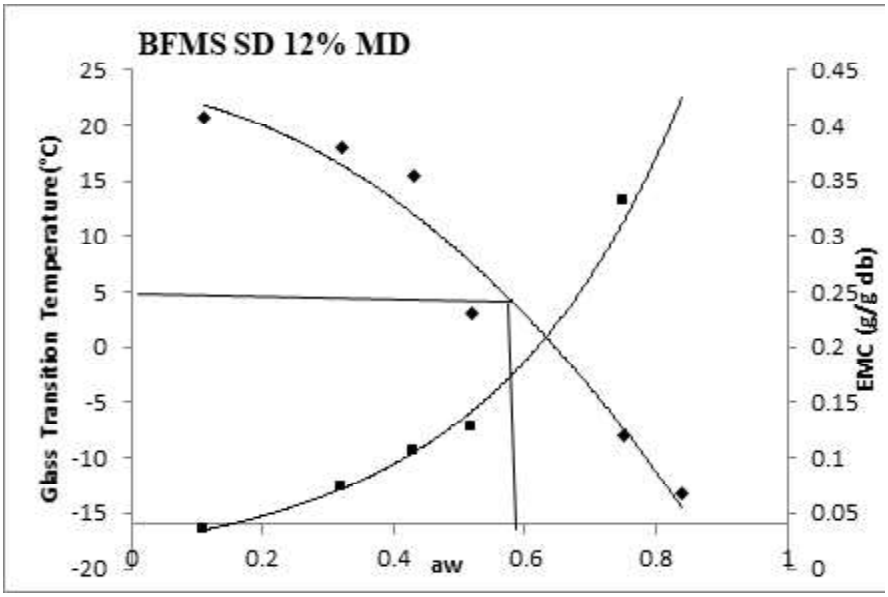

Fig. 6 Variation of glass transition temperature and equilibrium moisture content with water activity for spray dried avocado milkshake powder (BFMS-SD) produced with 12% maltodextrin (MD)

From the combined plot of Tg and EMC vs water activity (Figure 6) the critical water activity corresponding to different storage temperatures above its Tg value can be determined. This data is useful in determining the ideal storage conditions for the product. For example, if the powder has to be stored at 5°C its critical water activity from the plot is 0.6. From the plot for spray dried milk shake powder the critical water activity at 20°C is 0.1. For powders which have Tg above room temperatures the critical water activity is determined by extrapolating to the Tg curve at 25°C.

Both water activity and glass transition temperature have been widely used to evaluate storage stability. Roos (1995) reported that the plasticization of biosolids is a result of combined effects of water and temperature. According to the author, the prediction of food stability based only on sorption isotherms data is not enough, since certain physicochemical and structural processes such as stickiness, crispness, collapse, amorphous-to-crystalline transformations and the rates of non-enzymatic browning are not related to a monolayer value and they are better correlated to the glass transition temperature through plasticization by water or temperature. Thus, the use of state diagrams that indicate the material's physical state, combined with the sorption isotherms, helps in the prediction of food stability, regarding to its physical characteristics.

Several authors have coupled the data of sorption isotherms with those of glass transition temperature, in order to obtain the critical conditions for food storage (Moraga et al. 2004, 2006). The critical water content/ water activity is the value at which the glass transition temperature of the product is equal to the room temperature. Above this temperature, the amorphous powders are susceptible to deteriorative changes like collapse, stickiness and caking, resulting in quality loss.

Conclusions

Among all the samples prepared by spray drying with varying levels of MD, avocado milkshake powder with 12% MD was found to be the best without any stickiness and bitter taste. The colour was also acceptable for this sample. GAB model was found to be adequate (R^2=0.99) to describe the experimental sorption data obtained for the spray-dried avocado milkshake powder. The glass transition temperature was determined for different water activities, in which an increase in moisture content caused a significant decrease in the glass transition temperature. The data for Tg fitted well with the Gordon-Taylor model. (R^2=0.89). The addition of maltodextrin increased the Tg and consequently contributed to the stability of the powder. These data could be used to assist the proper spray drying operational conditions with respect to stickiness and storage behaviour of the milk shake of a fat rich fruit like avocado. Likewise, powder with 12% maltodextrin was more stable at a higher relative humidity (critical water activity 0.6), which contributes to the prevention of caking and diffusion controlled deteriorative processes. This combined plot of Tg and sorption is a better tool for determining the ideal storage conditions for the product.

References

Adhikari B, Howes T, Bhandari BR, Troung V (2004) Effect of addition of maltodextrin on drying kinetics and stickiness of sugar and acid-rich foods during convective drying: experiments and modelling. J Food Eng 62: 53-68

AOAC (2005). Official methods of analysis of the association of official analytical chemists international. USA: Maryland

Bhandari BR, Hartel RW (2005) Phase transitions during food powder production and powder stability

Brunauer S, Emmett PH, Teller E (1938) Adsorption of gases in multimolecular layers. J Am Chem Soc 60: 309-319

Cano-Chauca, M, Stringheta, PC, Ramos AM, Cal-Vidal J (2005) Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. Innov Food Sci Emerg Technol 6: 420-428

DorantesL, Parada L, Ortiz A (2004) Avocado: post-harvest operation. AGST/FAO.

Duester KC (2000) Avocados a look beyond basic nutrition for one of nature's whole foods. Nutr Today 35: 151-157

Gabas AL, Telis VRN, Sobral PJA, Telis-Romero J (2007) Effect of maltodextrin and arabic gum in water vapor sorption thermodynamic properties of vacuum dried pineapple pulp powder. J Food Eng 82: 246-252

Gordon M,TaylorJS(1952)Ideal copolymers and the second order transitions of synthetic rubbers. I. Non crystalline copolymers. J Appl Chem 2: 493-500

Johari GP, Hallbrucker A, Mayer E (1987) The glass–liquid transition of hyper quenched water. Nat 330: 552-553

KhalloufiS, El MaslouhiY, Ratti C (2000) Mathematical model for prediction of glass transition temperature of fruit powders. J Food Sci 65: 842-848

KurozawaLE, Park KJ, Hubinger MD (2009) Effect of maltodextrin and gum arabic on water sorption and glass transition temperature of spray dried chicken meat hydrolysate protein. J Food Eng 91: 287-296

LewickiPP(1997) The applicability of the GAB model to food water sorption isotherms. Int J Food Sci Technol 32: 553-557

Mara RighettoA, Maria Netto F (2005) Effect of encapsulating materials on water sorption, glass transition and stability of juice from immature acerola. Int J Food Prop 8: 337-346.

Moraga G, Martýnez-Navarrete N, Chiralt A (2004) Water sorption isotherms and glass transition in strawberries: influence of pretreatment. J Food Eng 62: 315-321

Moraga G, Martínez-Navarrete N, Chiralt A (2006) Water sorption isotherms and phase transitions in kiwifruit. J Food Eng 72: 147-156

Ozmen L, Langrish TAG (2002) Comparison of Glass Transition Temperature and Sticky Point Temperature for Skim Milk Powder. Drying Technol20: 1177-1192

Pérez-Alonso C, Beristain CI, Lobato-Calleros C, Rodríguez-Huezo ME, Vernon-Carter EJ (2006) Thermodynamic analysis of the sorption isotherms of pure and blended carbohydrate polymers. J Food Eng 77: 753-760

Rahman MS (2010) Food stability determination by macro–micro region concept in the state diagram and by defining a critical temperature. J Food Eng 99: 402-416

Rahman MS (2007) Handbook of Food preservation. CRC press.

Rainey C, Affleck M, Bretschger K, Alfin-Slater RB (1994) The California avocado: a new look. Nutr Today 29: 23-27

Ranganna S (2004) Handbook of analysis and quality control for fruit and vegetable products. Tata McGraw-Hill Education.

Reineccius GA (1991) Carbohydrates for flavor encapsulation. Food Technol 45: 144-146

Roos YH (1995) Food components and polymers. Phase transitions in foods, 109-156

Roos Y, Karel M (1991) Amorphous state and delayed ice formation in sucrose solutions. Int J Food Sci Technol 26: 553-566

Shivakumar KM, Chetana R, Reddy SY (2012) Preparation and properties of encapsulated fat powders containing speciality fat and ω/PUFA-rich oils. Int J Food Prop 15: 412-425

Shrestha AK, HowesT, Adhikari BP, Bhandari BR (2007) Water sorption and glass transition properties of spray dried lactose hydrolysed skim milk powder. Lwt-Food Sci Technol 40: 1593-1600

Silva MA, Sobral PJA, Kieckbusch TG (2006) State diagrams of freeze-dried camu-camu (Myrciariadubia (HBK) Mc Vaugh) pulp with and without maltodextrin addition. J Food Eng 77: 426-432.

Xiong H, Tang HM, Xiong XQ, Zheng WW (2004) Nutritional and functional properties of cream powder of imitation mother's dairy [J]. China Dairy Industry, 8 www.cnki.com.cn

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RESEARCH ARTICLE

Influence of red grape pomace powder on physiochemical, antioxidant, nutritional, microbial, and sensory properties of probiotic yoghurt

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Abstract: In this study, we investigated the effect of 2 and 4 % grape pomace powder (GPP) on the physiochemical, nutritional, microbial and sensory properties of probiotic yoghurt (contains *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) during the storage period every 7-day intervals (for 28 days). The results revealed that the pH, fat, protein, syneresis and sensory scores of samples decreased with elevation the grape pomace powder (GPP) level and also storage time. Ash, fiber, energy, total flavonoid, antioxidant activity, total phenol and the counting (log cfu/mL) of probiotic microorganisms decreased during storage, but the treatments with higher GPP had higher ash, fiber, energy, total flavonoid, antioxidant activity, total phenol content and probiotic counts. In converse, the acidity and viscosity increased with time storage and increase the GPP level. The probiotic yoghurt supplanted with 4% GPP in 0 day had the highest ash (1.2%), fiber (0.75%), energy (73.8 kcal/g), total flavonoid (4.9 mg Rutin equivalents/g) and antioxidant activity (42.4%). The highest viable count at the end of storage period (28 day) was obtained for probiotic yoghurt containing 4% GPP (8.03 log cfu/mL) and the lowest for control (6.2 log cfu/mL). Also the highest organoleptical scores belonged to yoghurt supplemented with 2% GPP at 0 day. The results of this research showed that grape pomace can be used in the production of probiotic yoghurt.

Keywords: Antioxidant, Grape pomace, Organoleptic, Probiotic yoghurt, Protein, Viscosity

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Introduction

Yoghurt is a fermented dairy product that is fermented and acidified by addition of a starter culture containing fermenting lactic acid bacteria including *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*. Yoghurt has gained widespread consumer acceptance as a healthy food providing health benefits. The functionality of yoghurt increases with the addition of probiotic microorganisms. Probiotics are living microorganisms having beneficial effect on host health (Pereira and Gibson 2002). The most commonly used probiotic microorganisms in dairy food are *L. acidophilus* and *B. bifidum*, which are chosen for their nutritive, therapeutic, and symbiotic characteristics (Hull et al. 1984, Gomes and Malcata 1999). Prebiotics are non-digestible carbohydrate substrates in the diet that are the preferred foods for *Bifidobacteria* and *Lactobacilli* and result in their increased number in the large intestine (Gibson and Roberfroid 1995). Grape (*Vitis* sp., Vitaceae) is one of the world's largest fruit crops, with an approximate annual production of 58 million metric tons (Zhu et al. 2014, Llobera and Cañellas 2007, Schieber et al. 2001). Winemaking process uses a considerable amount of fresh grape generating a huge mass of solid by-products. This by-product, usually referred to as grape pomace (GP), is generated after destemming and pressing grapes and is composed of grape seeds and skins (Cheng et al. 2010). In a study chemical composition of grape pomace evaluated. With regard to the compounds with functional properties, higher values of total dietary fiber (46.17 g/100 g), insoluble fiber (36.4 g/100 g), carbohydrate (29.2 g/100 g), protein (8.49 g/100 g), lipids (8.16 g/100 g), energy (224 kcal/100 g), vitamin C (26.25 mg /100 g), and anthocyanins (131 mg/100 g) were found. The minerals iron, potassium, zinc, manganese, and calcium were present in higher concentrations (Sousa et al. 2014). As mentioned above, grape pomace is a rich source of polyphenols and fibers. Agte et al. (2010) demonstrated prebiotic activity in grape varieties and hybrids that showed from 21.2 to 72.5% of the activity of fructo-oligosaccharide (FOS) used as a standard prebiotic. In this sense, Dairy products, such as yoghurts, are interesting foods in which grape pomace may be added, due to its positive effect on probiotic bacteria and consumers that prefer natural fibers instead of synthetic ingredients (Ramos et al. 2017). Some researchers studied the effect of the grape (juice, skin, seed, pomace, flour,

oligofructose, extract) on the physiochemical, textural, sensory, nutritional, antioxidant and microbiological properties of yoghurt (Oztürk and Oner 1999, Karnopp et al. 2017, Dos Santos et al. 2017, Hervert-Herna´ndez et al. 2009, Da Silva et al. 2017, Karaaslan et al. 2011, Chouchouli et al. 2013, Lachman et al. 2013, Gil-Sánchez et al. 2017). Also, consumption of fruits, prebiotics and yoghurt in combination has a potential to provide extra nutritional-physiological value that involve in synergetic effect on health such as cancer and cardiovascular disease (Espírito-Santo et al. 2010, Kourkoutas et al. 2006, Sendra et al. 2008, Karaaslan et al. 2011). Although the utilization of grape pomace in yoghurt was already the target of investigations (Marchiani et al. 2016), there is no comprehensive information about the physiochemical, microbial and sensory properties of fortified probiotic yoghurt with red pomace powder. Hence, we evaluated the combined effect of grape pomace powder and probiotic bacteria, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on some quality and traits of yoghurt and produced a yoghurt formulation based on sensorial, nutritional, and functional perspectives. Therefore, the objectives of this study were to investigate grape pomace potential as a functional ingredient for yoghurt production, and to evaluate the chemical, physical, microbial and sensory properties of the product during refrigerated storage (4 ºC).

Materials and Methods

Materials

Whole pasteurized cow milk used to prepare yoghurt formulations. Lyophilized pouches of commercial ABY culture (containing *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* BB-12, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) and Y culture (containing commercial yoghurt starter culture, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) supplied by Christian Hansen (Horsholm, Denmark). The cultures maintained according to manufacturer's instructions at -18 °C until use. Red wine grape pomace obtained from an industry of fruit pulp locate in the city of Gorgan (Iran). The pomace of grape fruit dried in oven under air flow at 60 °C until constant weight. The dried residue reduced to fine powder in a bimby processor. In order to make the mixture of the powder into the reconstituted milk easier, the particle size standardized to less than 0.42 mm, measured through sieves. The powder stored in clapped glass bottles and kept under refrigeration at 4 °C until use.

Yoghurt production

Milk standardized to 14% (w/v) total solids with skimmed milk powder. The mixture was pasteurized at 85 °C for 30 min and cooled to 43 °C and inoculated with the standard yoghurt culture (Y) and the working cultures of *L. acidophilus* and *B. bifidum* at the level of 1% (v/v). For the preparation of fruit incorporated yoghurt, 2 and 4 % (w/v) red grape pomace powder added to the milk before adding skim milk powder during the standardization. The mixture was incubated at 42 °C until the pH reached 4.5. After the fermentation, the samples were transfered into a refrigerator at 4°C and then stored at 4°C for 28 days. pH, total titratable acidity, protein, fat, ash, total dietary fiber, energy, syneresis, viability of probiotic organisms, total phenolic content, total flavonoid content, antioxidant activity, viscosity and sensory properties were determined during the storage period every 7-day intervals.

Chemical and nutritional attributes

pH values of the samples measured using a pH meter. The titratable acidity (Dornic, °D) determined after mixing 10 mL of sample with 10 mL of distilled water and titrating with 0.1 N NaOH using phenolphethalein as indicator (Ahmadi et al. 2012). Ash content was detected according to Sowbhagya et al. (2007). Fat was measured gravimetrically by extraction with diethyl ether using a Soxhlet apparatus (AOAC 1990). Nitrogen content was detected by Kjeldahl method (Ayadi et al. 2009). Total dietary fiber content determined with using of the Megazyme International total DF assay (Sun-Waterhouse et al. 2010). The total energy was measured based on the energy nutrient results obtained using the conversion factors of Atwater, as described by Sousa et al. (2014) considering 4 kcal/g for carbohydrate, 4 kcal/g for protein, and 9 kcal/g for lipids. Syneresis determined by measuring the volume of separated whey (mL whey/ 100 mL yoghurt) after 30 min at room temperature (Abd El-Salam et al. 1991).

Total phenolic content

Total phenolic content was measured according to Zheng and Wang (2001) by using Folin–Ciocalteu reagent. The absorbance measured with a UV–ViS Spectrophotometer at 765 nm. The absorbance values were converted to the total phenolics and were expressed as µg gallic acid equivalents per gram sample (µg GAE/g).

Total flavonoid content

The amount of total flavonoid content (TFC) in the extracts was detected spectrophotometrically according to Djeridane et al. (2006). This method depends on the formation of a complex flavonoid aluminum, having the maximum absorbance at 430 nm. Rutin was used to make a calibration curve. TFC expressed as mg Rutin equivalents per g.

Antioxidant activity

Antioxidant activites of yoghurt samples by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) inhibition were determined. Yoghurt water extract (250 µL) was added into 3 mL of 60 µM DPPH in ethanol. The decrease in absorbance monitored at 517 nm until a

constant reading obtained. The readings compared with the control which contained distilled water (250 µL) (Apostolidis et al. 2007). The inhibition percentage calculated as follows:

% Inhibition=(A control"A sample)×100 / A control

Viscosity

The apparent viscosity of samples was measured using a rotary Brookfield Viscometer (RVDV2, Brookfield, MA, USA) with RV4 spindle. Before starting the test, all samples were kept at 7°C in constant conditions to remove any stress or change in their texture. Viscosity of each treatment was carried out at 80 rpm shear stress during 60 s. The test type was as single point (Trachoo and Mistry 1998).

Probiotic Count

MRS-bile agar medium used for the selective enumeration of *L. acidophilus* and bifidobacteria (Sohrabvandi et al. 2012). The plates incubated anaerobically at 37°C for 72 h. Anaerobic conditions produced using the GasPack system. Number of probiotics calculated and expressed as cfu/mL (Shafiee et al. 2010).

Sensory Analysis

Yoghurt samples were subjected into a sensory evaluation with 10 untrained panelists. The yoghurt samples were served in white plastic pots in individual booths under light exposure. The sensory evaluation carried out using a five-point hedonic scale (5- like very much to 1- dislike very much) based on acceptance to the product in order to evaluate the degree of likeliness for selected quality attributes i.e. flavor, appearance, consistency, taste, and overall acceptability (Senadeera et al. 2018).

Statistical analysis

The data obtained from the measurements were subjected to analysis of variance (ANOVA) to determine the significant differences among the samples, and the values were compared using the Duncan's test defined at Pd"0.05. All measurements were carried out in triplicate and reported as the mean±SD. The data analysis was performed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Effect of incorporation of grape pomace powder on physiochemical, antioxidant and nutritional properties of probiotic yoghurt

Chemical and nutritional properties of different yoghurt samples during 28 days storage is presented in Table 1. The pH of all yoghurt samples decreased throughout storage period, while the acidity increased. This was due to the growth of lactic acid bacteria and produced the lactic acid (Rasic et al. 1978). The values of pH and acidity recorded in this study were consistent with the results obtained by Mahmood et al. (2008) and Tarakci (2010).

The fat and protein of samples were decreased with increase the grape pomace powder (GPP) and also during storage time, while

Table 1 Physiochemical and nutritional parameters of different yoghurt formulations during storage period (mean ± SD)

	Day Treatment	pH	Acidity (°D)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Energy (kcal/g)	Syneresis) (mL whey/ 100 mL yoghurt
control	0	4.8±0.1^{g}	80±1.7^{a}	3.2±0.1^{a}	3.4±0.2^{f}	0.8±0.1^{a}	0.07±0.01^{a}	70±1.1^{ab}	50±2.2^{k}
	7	4.6±0.1^{fg}	82.0±2^{b}	3.2±0.2^{a}	3.4±0.2^{f}	0.8±0.1^{a}	0.07±0.01^{a}	70±2.1^{ab}	49.9±2.1^{k}
	14	4.6±0.2^{fg}	83.0±3.2^{b}	3.15±0.2^{a}	3.3±0.1^{ef}	0.78±0.2^{a}	0.07±0.01^{a}	69.6±2.2^{ab}	46.7±1.7^{j}
	21	4.4±0^{efg}	85.0±2.3^{c}	3.12±0.1^{a}	3.3±0.1^{ef}	0.78±0.12^{a}	0.065±0.01^{a}	69.2±1.9^{a}	42.8±1.8^{g}
	28	4.3±0.07^{defg}	86.0±4^{cd}	3.1±0.23^{a}	3.28±0.13^{def}	0.77±0.06^{a}	0.06±0^{a}	69.0±1.7^{a}	39.8±1.9^{f}
2% GPP	0	4.7±0.3^{g}	83.0±1.1^{b}	3.0±0.3^{a}	3.2±0.3^{cde}	1.08±0.21^{bcd}	0.38±0.08^{b}	72±2.3^{def}	44.3±2^{i}
	7	4.3±0.11^{defg}	87.0±2.2^{d}	3.0±0.24^{a}	3.18±0.21^{cde}	1.07±0.04^{bc}	0.38±0.06^{b}	72±3.4^{def}	43.8±3^{h}
	14	4.0±0.21^{bcde}	90.0±3.4^{e}	2.9±0.34^{a}	3.16±0.17^{bcde}	1.06±0.06^{bc}	0.38±0.02^{b}	71.7±0.8^{de}	43.2±0.9^{g}
	21	3.8±0.14^{abcd}	93.0±2.4^{f}	2.9±0.17^{a}	3.13±0.16^{bcd}	1.05±0.09^{b}	0.37±0.03^{b}	71.2±4.1^{cd}	39.2±1.5e^{i}
	28	3.6±0.32^{abc}	97.0±2.8^{g}	2.8±0.14^{a}	3.1±0.2^{bc}	1.04±0.1^{b}	0.37±0.03^{b}	70.5±2.2^{bc}	36.4±1.2^{d}
4% GPP	0	4.5±0.25^{efg}	87.0±1.6^{d}	2.9±0.15^{a}	3.1±0.2^{bc}	1.2±0.12^{e}	0.75±0.04^{c}	73.8±1.4^{h}	31.5±0.8^{c}
	7	4.1±0.30^{cdef}	92.0±1.9^{f}	2.9±0.09^{a}	3.1±0.1^{bc}	1.2±0.1^{e}	0.75±0.02^{c}	73.3±1.3^{gh}	31.3±0.7^{bc}
	14	3.8±0.42^{abcd}	96.0±3.6^{g}	2.8±0.08^{a}	3.0±0.1^{ab}	1.17±0.07^{de}	0.75±0.01^{c}	73±1.7f^{gh}	31.1±0.6^{ab}
	21	3.5±0.33^{ab}	101.2±3.8^{h}	2.8±0.1^{a}	3.0±0.13^{ab}	1.15±0.05^{cde}	0.74±0.04^{c}	72.6±1.4^{efg}	30.9±0.5^{b}
	28	3.4±0.18^{a}	105.4±2.5^{i}	2.7±0.1^{a}	2.9±0.08^{a}	1.13±0.03^{bcde}	0.74±0.01^{c}	72.1±2.2^{def}	30.2±0.8^{a}

Values are given as mean ± SD.
Different letters in the same column indicate significant differences ($p < 0.05$).

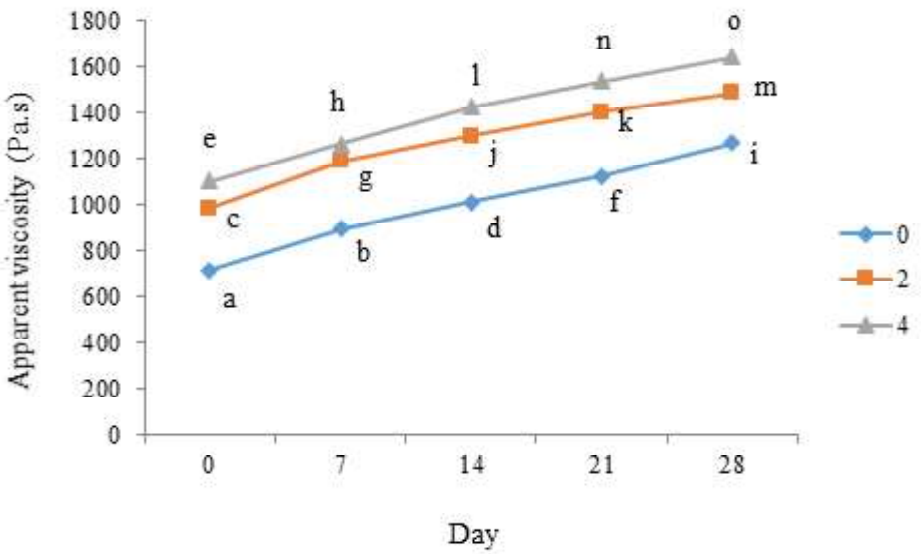

Fig 1. Variation in the viscosity of different probiotic yoghurt formulations during storage period. Values are given as mean ± SD. Different letters indicate significant differences (P < 0.05).

Table 2 Total phenol and flavonoid contents and Antioxidant activity of different probiotic yoghurt formulations during storage period (mean ± SD)

Treatment	Day	Total phenolic (µg GAE/g)	Total flavonoid (mg Rutin equivalents/g)	Antioxidant activity (%)
control	0	9.21±0.70^{ab}	1.0±0.03^{c}	21.40±1.30^{e}
	7	9.30±0.40^{c}	0.90±0.04^{bc}	18.10±1.10^{d}
	14	9.12±0.30^{a}	0.80±0.05^{b}	16.70±1.00^{c}
	21	9.18±0.28^{ab}	0.60±0.02^{a}	14.30±0.80^{b}
	28	9.16±0.32^{ab}	0.50±0.01^{a}	12.20±0.60^{a}
2% GPP	0	12.82±0.21^{d}	2.44±0.10^{g}	28.50±2.10^{i}
	7	13.10±20.22^{e}	2.10±0.10^{f}	26.10±2.00^{g}
	14	12.76±0.41^{d}	1.80±0.09^{e}	23.40±1.40^{f}
	21	12.70±0.10^{cd}	1.70±0.06^{e}	21.00±1.00^{e}
	28	12.60±0.18^{c}	1.50±0.12^{d}	18.30±0.50^{d}
4% GPP	0	16.40±0.25^{f}	4.90±0.22^{l}	42.40±1.20^{l}
	7	16.70±0.30^{g}	4.60±0.25^{k}	37.60±2.30^{k}
	14	16.60±0.27^{g}	4.20±0.27^{j}	33.20±2.10^{j}
	21	16.40±0.30^{f}	3.60±0.30^{i}	27.50±1.40^{h}
	28	16.30±0.27^{f}	3.20±0.26^{h}	23.10±1.00^{f}

Values are given as mean ± SD.
Different letters in the same column indicate significant differences (P < 0.05).

the syneresis was decreased. The differences between protein of treatments were not significantly difference (p>0.05). Ash, fiber and energy decreased during storage, but the treatments with higher GPP had higher ash, fiber and energy contents. The yoghurt supplemented with 4% GPP in 0 day had the highest ash (1.2%), fiber (0.75%) and energy (73.8 kcal/g) (Table 1). This result is due to low fat and protein, and high ash, fiber and energy content of grape pomace. Similarly, in research carried out by Karnopp et al. (2017), grape skin flour (GSF) increased the ash and total fiber contents of yoghurts that were in parallel with our findings. Conversely, Da Silva et al. (2017) reported that pH, titratable acidity, ash, fat and moisture content of probiotic yoghurt supplemented with 1.5 and 3.0 g L^{-1} of grape extract that stored at 4 °C were not significantly different (P < 0.05).

Syneresis is the leakage of liquid from yoghurt. Syneresis is one of the key quality parameters for yoghurt. Higher level of syneresis revealed that yoghurt is of low quality (Lee and Lucey 2010). The syneresis of yoghurts were affected significantly (P<0.05) by both grape pomace concentration and storage time and the changes are presented in Table 1. Syneresis values of different types of probiotic yoghurt varied from 30.2 to 50.0%. This result was matched with (Tarakci and Kucukoner 2003). In the first day, the highest mean value (50%) of syneresis was related to control and the lowest mean value (31.5 %) in sample containing 4% GPP. The addition of fruit pomace powder caused

a decrease of syneresis in all samples of yoghurts and the differences between the control and these samples were statistically significant (P<0.05). It could be related to the capacity to absorb water by solids (fiber, carbohydrate and protein) that present in pomace powder which leads to a decrease of syneresis (Mahmood et al. 2008). But also controversial results also reported. For example, Da Silva et al. (2017) noted that syneresis increased with high concentration of grape extract. While Dos Santos et al. (2017) observed no differences for syneresis in yoghurt samples fortified with Pinot Noir grape juice and concentrated grape skin extract.

Since grape pulp contains a wide variety of phenolic compounds (Chung et al. 1998), its regular consumption reduces the risk of diseases such as cancer and cardiovascular diseases (Jang et al. 2010). Total phenolic, total flavonoid contents and antioxidant activity of probiotic yoghurt samples are shown in Table 2. There were significant differences in the total phenolic, total flavonoid contents and antioxidant activity of the samples (P <0.05). All these factors were decreased throughout storage period. But also these factors for supplemented yoghurt samples were significantly (p<0.05) higher than control samples. In a study, yoghurts supplemented with red grape and callus extracts displayed high phenolic and anthocyanin content and thus exhibited higher antioxidant power compared to yoghurts containing chardonnay extracts and control samples. The storage time significantly affected the free radical scavenging capacity of the yoghurts. The yoghurts supplied with grape callus extract displayed the greatest antioxidant power on the first day of storage compared to all the assayed samples (Karaaslan et al. 2011).

In our study, the highest total flavonoid (4.9 mg Rutin equivalents/ g) and antioxidant activity (42.4%) recorded for yoghurt enriched with 4% GPP at 0 day that had significant differences with other treatments (p<0.05). But the higher total phenolic (16.7 µg GAE/ g) content was obtained for yoghurt enriched with 4% GPP at 7 day. The higher values of these factors were for enriched probiotic yoghurts with grape pomace powder compared to control, it may be due to the high concentrations of antioxidant compounds of grape pomace (Leong and Shui 2002) have observed the highest activity using DPPH. Similar studies describe that the antioxidant activity of yoghurts was enhanced by the presence of natural extracts, for example, in studies with yoghurts fortified with white and red dragon fruit (Zainoldin and Baba 2009), grape seed (Chouchouli et al. 2013) or with wild blackberry (Martins et al. 2014) extracts. Generally, the development of dairy foods containing polyphenols and fibers from grape pomaces are a technological trend that can improve the nutritional and functional value of foods (Dos Santos et al. 2017, Lachman et al. 2013).

The viscosities of liquid and semisolid foods have a large impact on their quality properties (Karaman et al. 2014). Figure 1 is presented the viscosity of yoghurt samples during storage time. It was obvious that the apparent viscosity of all fruit yoghurts increase in proportion to increase percentage of pomace powder addition and also storage time. The lowest and highest viscosity recorded for control sample at 0 day (714 Pa.s), and yoghurt supplemented with 4% GPP at 28 day storage (1644 Pa.s), respectively (Figure 1). In a study, purple grape juice (PGJ) increased the viscosity of yoghurt, but the combinations 50% grape skin flour (GSF) and 50% oligofructose (OLI) and 66.7% GSF, 16.7% OLI and 16.7% PGJ presented the lowest mean values for viscosity (Karnopp et al. 2017). A similar trend was found by Öztürk and Öner (1999) for yoghurt containing concentrated grape juice. The fibers in Grape acted as stabilizer, forming colloidal solution (three dimensional network) that prevented water mobility, and so yielding high values of viscosity (Cruz et al. 2013).

Effect of incorporation of grape pomace powder on microbial counts of probiotic yoghurt

A sufficient number of viable microorganisms must be present throughout the entire shelf life of the product in order to produce therapeutic benefits. Although there is no world-wide agreement on the minimum of viable probiotic cells per gram or milliliter of probiotic product until the time of consumption, generally, the values of 10^6 and 10^7-10^8 cfu/mL or cfu/g have been accepted as the minimum and satisfactory levels, respectively (Korbekandi et al. 2011, Tamime et al. 2005, Ahmadi et al. 2012, Mortazavian et al. 2008). Usually due to high acid content, presence of bacteriocins, and fermentation conditions, the survival ability of probiotic bacteria in yoghurt decreased before consumption (Ferdousi et al. 2013). Therefore, utilization of prebiotic compounds is a common method in order to increase the survival ability of these bacteria at the consumption time (Pharmaceutiques 1995). Prebiotic activity of grape previously reported (Agte et al. 2010). Viability (log cfu/mL) of probiotic microorganisms in different treatments during refrigerated storage (4°C) for 28 days is presented in Figure 2. The probiotic microorganisms (log cfu/ mL) in different treatments during refrigerated storage (4°C) decreased by time, but also this decrease was lower in samples contain GPP. Although counts of probiotic bacteria decreased gradually during storage time, yoghurt samples held probiotic values of 10^6 log cfu/mL until day 28. Vinderola and Reinheimer (1999) also stated that probiotic microflora counts decrease during storage. The rate of this loss in cell viability depended on the yoghurt type and the use of lactic starter. In current study the highest viable counts at the end of storage period (28 day) were obtained for probiotic yoghurt contain 4% GPP (8.03 log cfu/mL) and the lowest for control (6.2 log cfu/mL) (Figure 2). In a research carried out by Da Silva et al. (2017), bacterial strains were found in at least 10^9 cfu/g of yoghurt showing the possibility of probiotic yoghurt production with grape extract that overlapped with current study as yoghurt containing 4% GPP had 8.03 log cfu/mL at the end of storage time (Figure 2). Also Ehsani et al. (2015)

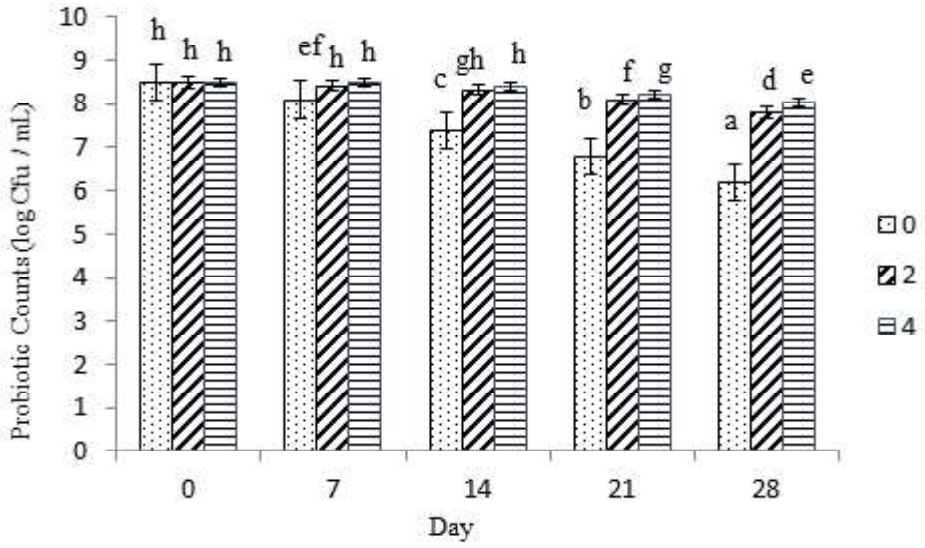

Fig 2. Viability (log cfu/mL) of probiotic microorganisms in different yoghurt samples during refrigerated storage (4°C) for 28 days. Values are given as mean ± SD. Different letters in the same column indicate significant differences ($P < 0.05$).

Table 3 Sensory analysis of the probiotic yoghurt treatments using score methodology

Treatments	Day	Appearance	Taste	Consistency	flavor	Overall acceptability
control	0	5.0±0.20^{c}	4.5±0.20^{e}	4.7±0.10^{gh}	4.7±0.20^{cde}	4.7±0.00^{g}
	7	4.8±0.10^{bc}	4.3±0.10^{d}	4.5±0.20^{ef}	4.5±0.10^{bcde}	4.4±0.00^{f}
	14	4.5±0.12^{abc}	4.0±0.08^{c}	4.2±0.08^{bc}	4.2±0.20^{abc}	4.2±0.10^{de}
	21	4.3±0.22^{abc}	3.8±0.06^{b}	4.0±0.06^{a}	4.0±0.00^{ab}	4.0±0.10^{bc}
	28	4.2±0.14^{ab}	3.7±0.06^{ab}	4.0±0.05^{a}	3.8±0.08^{a}	3.8±0.20^{a}
2% GPP	0	50±0.20^{c}	4.8±0.40^{f}	4.9±0.17^{i}	5.0±0.07^{e}	4.9±0.10^{h}
	7	4.7±0.30^{bc}	4.5±0.30^{e}	4.8±0.14^{hi}	4.9±0.20^{e}	4.7±0.30^{g}
	14	4.6±0.24^{bc}	4.2±0.10^{d}	4.6±0.15^{fg}	4.7±0.10^{cde}	4.4±0.40^{f}
	21	4.5±0.26^{abc}	4.0±0.00^{c}	4.4±0.27^{de}	4.3±0.30^{abcd}	4.3±0.00^{ef}
	28	4.3±0.27^{abc}	3.8±0.08^{b}	4.3±0.18^{cd}	4.0±0.20^{ab}	4.2±0.10^{de}
4% GPP	0	4.7±0.28^{bc}	4.6±0.12^{ef}	4.8±0.16^{hi}	4.8±0.20^{de}	4.7±0.10^{g}
	7	4.5±0.21^{abc}	4.3±0.14^{d}	4.6±0.31^{fg}	4.7±20^{cde}	4.6±0.30^{g}
	14	4.3±0.18^{abc}	4.0±0.09^{c}	4.5±0.24^{ef}	4.5±0.10^{bcde}	4.4±0.10^{f}
	21	4.1±0.19^{ab}	3.8±0.10^{bc}	4.3±0.20^{cd}	4.2±0.10^{abc}	4.1±0.20^{cd}
	28	3.8±0.18^{a}	3.6±0.10^{a}	4.1±0.11^{ab}	3.9±0.30^{a}	3.9±0.20^{ab}

Values are given as mean ± SD.
Different letters in the same column indicate significant differences ($P < 0.05$).

noted that the viability of both probiotic bacteria were significantly greater in the treatments containing artichoke extract compared to control yoghurts during storage period of 28 days. Similarly, Mocanu et al. (2010) observed that highest number of probiotic bacteria at the end of refrigerate storage, belongs to yoghurt containing mixture of bilberry and liquorice extract compared to control yoghurt.

The antioxidant compounds in grapes can affect the survival of probiotics in yoghurt. But in some studies, the authors did not find an inhibitory effect (Hervert-Herna´ndez et al. 2009, Chouchouli et al. 2013). It is possible that phenolic compounds are transformed into more active derivatives (e.g. aglycones) under certain conditions, which might enhance starter culture activity (Sun-Waterhouse et al. 2013). In our study, after 28 days of storage, in all cases (even control treatment) the viable numbers of probiotics remained in values higher than 10^6 cfu/mL that is necessary to confer the probiotic character of the products.

Effect of incorporation of grape pomace powder on sensory properties of probiotic yoghurt

Fruit mixes improve the nutritional value and the taste of yoghurt, and fruit enhancement plays a considerable role in yoghurt consumption and sales (Kailasapathy et al. 2008). Sensory properties of yoghurt samples prepared in this study are shown in Table 3. Sensory scores decreased during storage period. This could be attributed to the development of acidity. Similar results reported by Cakmakci et al. (2012) about probiotic banana

yoghurts. Regarding appearance attribute there was not significant differences between samples (Table 3).

The lowest appearance (3.8) and taste (3.6) scores were obtained for sample containing 4% GPP at 28 day storage, and the lowest consistency (4.0), flavor (3.8) and overall acceptability (3.8) scores were obtained for control sample at the end of storage period. While the highest sensory scores belonged to yoghurt supplemented with 2% GPP at 0 day (5.0, 4.8, 4.9, 5.0 and 4.9 respectively). In our study, the addition of grape pomace has no negative effect on the color of yoghurt samples. In parallel with our results, Dos Santos et al. (2017) observed the higher sensory scores for flavor, color, and overall acceptability about fermented goat milk added with grape pomace extract. In the study done by Chouchouli et al. (2013) the fortification of full-fat and non-fat yoghurts with grape seed extracts did not cause major defects in consistency, color and flavor compared to controls. Also in a research carried out by Da Silva et al. (2017), sensory attributes (color, flavor, taste, texture and appearance) of probiotic yoghurt supplemented with grape extract were acceptable by panelists. These findings proved that fruity yoghurt contains both the refreshing flavor and taste of fruit and beneficial effect of yoghurt (Mahmood et al. 2008). In current study, storage period had slightly effect on all attributes. According to this result grape pomace yoghurt at 2% has the most acceptability in point view of consumer (Table 3). Since grape pomace can be used in the production of probiotic yoghurt.

Conclusions

Grape pomace is a nutritious, but underused, by-product of winemaking containing fiber and antioxidants. Using a suitable production design, a new probiotic fortified yoghurt formulation with grape by-product could be optimized to enhance consumers' daily intake of antioxidants. The use of grape pomace powder in the development of value added food products will be a step toward making new functional foods, and partially solving waste management problem from wine production. As consumption of fruits, prebiotics and yoghurt in combination has a potential to provide extra nutritional-physiological value that involve in synergetic effect on health. The results of this study would provide an opportunity of dairy producer to develop a novel product in agreement with consumers' preferences. This research represents a new approach in the development of novel probiotic yoghurt with high nutritional quality for human and with great potential applications on food industry.

References

Abd El-Salam MH, El-Shibiniy S, Mahfuz MB, El-Dein HF, El-Atriby H, Antila V (1991) Preparation of whey protein concentrate from salted whey and its use in yoghurt. Dairy Res 58: 503-510

Agte V, Khetmalis N, Nilegaonkar S, Karkamkar S, Yadav S (2010) Prebiotic potential of 'juice grape' varieties and some hybrids. scientific and industri Res 69 : 850-854

Ahmadi E, Mortazavian AM, Fazeli MR, Ezzatpanah H, Mohammadi R (2012) The effects of inoculants variables on the physicochemical and organoleptic properties of Doogh. Int J Dairy Technol 2: 274-281

AOAC. Official methods of analysis. (1990). 16th ed. Assoc. Off. Anal. Chem., Arlington, VA. Apostolidis E, Kwon YI, Shetty K (2007) Inhibitory potential of herb, fruit, and fungal enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innovat Food Sci Emerg Technolo 8: 46-54

Ayadi MA, Abdelmaksoud W, Ennouri M, Attia H (2009) Cladodes from Opuntia ficus indica as a source of dietary fiber: Effect on dough characteristics and cake making. Industri Crop Product 30: 40-47

Cakmakci S, Cetin B, Turgut T, Gurses M, Erdogan A (2012) Probiotic properties, sensory qualities, and storage stability of probiotic banana yogurts. Turk J Vete Anim Sci 36: 231-237

Cheng VJ, Bekhit AEDA, Sedcole R, Hamid N (2010) The impact of grape skin bioactive functionality information on the acceptability of tea infusions made from wine by-products. Food Scie 75: 167–172

Chouchouli V, Kalogeropoulos N, Konteles SJ, Karvela E, Makris DP, Karathanos VT (2013) Fortification of yoghurts with grape (Vitis vinifera) seed extracts. LWT - Food Sci Technol 53: 522-529

Chung KT, Lu Z, Chou MW (1998) Mechanism of inhibition oftannic acid and related compounds on the growth of intestinal bacteria. Food Chem Toxicol 36: 1053–1060

Cruz AG, Cavalcanti RN, Guerreito LMR, Sant'Ana AS, Nogueira LC, Oliveira CAF, Bolini HMA (2013) Developing a prebiotic yogurt: rheological, physicochemical and microbiological aspects and adequacy of survival analysis methodology. Food Engine 114: 323–330

Da Silva DF, Teno´rio Junior NN, Gomes RG, Dos Santos Pozza MS, Britten M, Matumoto-Pintro PT (2017) Physical, microbiological and rheological properties of probiotic yogurt supplemented with grape extract. Food Sci Technol 54: 1608–1615

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006) Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem 97: 654–660

Dos Santos KMO, Oliveira IC, Lopes MAC, Cruz APG, Buriti FCA, Cabral LM (2017) Addition of grape pomace extract to probiotic fermented goat milk: the effect on phenolic content, probiotic viability and sensory acceptability. J Sci Food Agri 97: 1108–1115

Ehsani J, Mortazavian AM, Khomeiri M, Ghasem Nejad A (2015) Effects of artichoke (Synara scolymus L.) extract addition on microbiological and physio-chemical properties of probiotic yoghurt. 2015. J Microbiol, Biotechnol Food Sci 4: 536-541

Espírito-Santo AP, Silva RC, Soares FASM, Anjos D, Gioielli LA, Oliveira MN (2010) Açai pulp addition improves fatty acid profile and probiotic viability in yoghurt. Intern Dairy J 20: 415-422

Ferdousi R, Rouhi M, Mohammadi R, Mortazavian AM, Khosravi- Darani K, Homayouni Rad A (2013) Evaluation of probiotic survivability in Yogurt exposed to cold chain interruption. Iran J Pharmaceu Res 12: 139–144

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 71: 1401-1412

Gil-Sánchez I, Ayuda-Durán B, González-Manzano S, Santos-Buelga C, Cueva C, Martín-Cabrejas MA, Bartolomé B (2017) Chemical characterization and in vitro colonic fermentation of grape pomace extracts. J Sci Food Agri 97: 3433-3444 http://dx.doi.org/10.1002/jsfa.8197. in press.

Gomes AMP, Malcata FX (1999) Bifidobacterium spp. and Lactobacillus acidophilus: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trend Food Sci Technol 10: 139-157

Hervert-Herna´ndez D, Pintado C, Rotger R, GonˆI I (2009) Stimulatory role of grape pomace polyphenols on Lactobacillus acidophilus growth. Int J Food Microbiol 136: 119–122

Hull RR, Roberts AV, Mayes JJ (1984) Survival of Lactobacillus acidophilus in yogurt. Austra J Dairy Technol 39: 164-166
Jang IC, Jo EK, Bae MS, Lee HJ, Jeon GI, Park E, Yuk HG, Ahn GH, Lee SC (2010) Antioxidant and antigenotoxic activities of different parts of persimmon (Diospyros kaki cv. Fuyu) fruit. J Medicin Plan Res 4: 155-160
Kailasapathy K, Harmstorf I, Phillips M (2008) Survival of Lactobacillus acidophilus and Bifido bacterium animalis ssp. lactis in stirred fruit yogurts. Food Sci Technol 41: 1317-1322
Karaaslan M, Ozden M, Vardin H, Turkoglu H (2011) Phenolic fortification of yogurt using grape and callus extracts. LWT - Food Sci Technol 44: 1065-1072
Karaman S, Toker ÖS, Yüksel F, Çam M, Kayacier A, Doğan M (2014) Physicochemical, bioactive, and sensory properties of persimmon-based ice cream: technique for order preference by similarity to ideal solution to determine optimum concentration. J Dairy Sci 97: 97–110
Karnopp AR, Oliveira KG, De Andrade EF, Postingher BM, Granato D (2017) Optimization of an organic yogurt based on sensorial, nutritional, and functional perspectives. Food Chem 233: 401–411
Korbekandi H, Mortazavian AM, Iravani S (2011) Stability and technology of probiotic in fermented milks: In: probiotic and prebiotic foods: technology, stability and benefits to the human health, (Shah N, ed.) Nova Science Publishing Ltd, USA. pp. 131–169
Kourkoutas Y, Bosnea L, Taboukos S, Baras C, Lambrou D, Kanellaki M (2006) Probiotic cheese production using Lactobacillus casei cells immobilized on fruit pieces. J Dairy Sci 89: 1439-1451
Lachman J, Hejtmánkováa A, Hejtmánkováa K, Stepánka H, Pivec V, Skala O, Pribyl J (2013) Towards complex utilisation of winemaking residues: characterisation of grape seeds by total phenols, tocols and essential elements content as a by-product of winemaking. Indust Crop Produc 49: 445–453
Lee WJ, Lucey JA (2010) Formation and physical properties of yogurt. Asia-Austral J Anim Sci 23: 1127-1136
Leong LP, Shui G (2002) An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem 76: 69-75
Llobera A, Cañellas J (2007) Dietary fibre content and antioxidant activity of Manto Negro red Grape (*Vitis vinifera*): pomace and stem. Food Chem 101: 659-666
Mahmood A, Abbas N, Gilani AH (2008) Quality of stirred buffalo milk yoghurt blended with apple and bananafruits. Pakis J Agri Sci 45: 2 275- 279
Marchiani R, Bertolino M, Belviso S, Giordano M, Ghirardello D, Torri L, Zeppa G (2016) Yogurt enrichment with grape pomace: effect of grape cultivar on physicochemical, microbiological and sensory properties. Food Qual 39: 77–89
Martins A, Barros L, Carvalho AM, Santos-Buelga C, Fernandes IP, Barreiro F, Ferreira ICFR (2014) Phenolic extracts of Rubus ulmifolius Schott flowers: characterization, microencapsulation and incorporation into yogurts as nutraceutical sources. Food Func 5: 1091-1100
Mocanu D, Rotaru G, Botez E, Andronoid D, Nistor O (2010) Probiotic yogurt with medicinal plants extract: Physical–chemical, microbiological and rheological characteristics. J Agroalimen Proces Technol 16:469-476
Mortazavian AM, Ehsani MR, Azizi A, Razavi SH, Mousavi SM, Sohrabvandi S (2008) Viability of calcium alginate-microencapsulated probiotic bacteria in Iranian yogurt drink (Doogh) during the refrigerated storage period and under the simulated gastrointestinal conditions. Austral J Dairy Technol 63: 24-29
Öztürk BA, Oner MD (1999) Production and evaluation of yogurt with concentrated grape juice. J Food Sci 64: 530-532
Pereira DI, Gibson GR (2002) Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. Critic Rev Biochem Mole Biol 37: 259-281
Pharmaceutiques UDL (1995) Dietary modulation of the human colonie microbiota: Introducing the concept of prebiotics. J Nutri 125: 1401–1412
Ramos LR, Santos JS, Daguer H, Valese AC, Cruz AG, Granato D (2017) Analytical optimization of a phenolic-rich herbal extract and supplementation in fermented milk containing sweet potato pulp. Food Chem 221: 950–958
Rasic JL, Kurmann JA (1978) Yogurt: Scientific Grounds, Technology, Manufacture and Preparations. Staempli Cie AG, Berne, Switzerland
Schieber A, Stintzing FC, Carle R (2001) By-products of plant food processing as a source of functional compounds. Recent developments (Review). Tren Food Sci Technol 12: 401-413
Senadeera SS, Prasanna PHP, Jayawardana NWIA, Gunasekara DCS, Senadeera P, Chandrasekara A (2018) Antioxidant, physicochemical, microbiological, and sensory properties of probiotic yoghurt incorporated with various Annona species pulp. Heliyon 4, https://doi.org/10.1016/j.heliyon.e00955.
Sendra E, Fayos P, Lario Y, Fernandez-Lopez J, Sayas-Barbera E, Perez-Alvarez JA (2008) Incorporation of citrus fibers in fermented milk containing probiotic bacteria. Food Microbiol 25: 13-21
Shafiee G, Mortazavian AM, Mohammadifar MA, Koushki MR, Mohammadi AR, Mohammadi R (2010) Combined effects of drymatter content, incubation temperature and final pH of fermentation on biochemical and microbiological characteristics of probiotic fermented milk. Afric J of Microbiol Res 4: 1265-1274
Sohrabvandi S, Mortazavian AM, Dolatkhah-Nejad MR, Bahadori Monfared A (2012) Suitability of MRS-bile agar for the selective enumeration of mixed probiotic bacteria in presence of mesophilic lactic acid cultures and yogurt bacteria. Iran J Biotechnol 10: 16-21
Sousa EC, Uchoa-Thomaz AMA, Carioca JOB, De Morais SM, De Lima A, Martins CG, Alexandrino CD, Ferreira PAT, Rodrigues ALM, Rodrigues SP, Silva JDN, Rodrigues LL (2014) Chemical composition and bioactive compounds of Grape pomace (*Vitis vinifera* L.), Benitaka variety, grown in the semiarid region of Northeast Brazil. Food Sci Technol (Campinas) 34: 135-142
Sowbhagya HB, Suma FP, Mahadevamma S, Tharanathan RN (2007) Spent residue fromcumin –a potential source of dietary fiber. Food Chem 104: 1220-1225
Sun-Waterhouse D, Teoh A, Massarotto C, Wibisono R, Wadhwa S (2010) Comparative analysis of fruit-based functional snack bars. Food Chem 4: 1369-1379
Tammime AY, Saarela M, Korslund Sondergaard A, Mistry VV, Shah NP (2005) In: Probiotic Dairy Products, (Tamime, AY, ed) Blackwell Publishing Ltd, UK
Tarakci Z (2010) Influence of Kiwi Marmalade on the Rheology Characteristics, Color Values and Sensorial Acceptability of Fruit Yogurt. Kafkas Universitesi Veteriner Fakultesi Dergisi 16: 173-178
Tarakci Z, Kucukoner E (2003) Physical, chemical, microbiological and sensory characteristics of some fruit-flavored yoghurt, YYU Vet Fak Dergisi 14: 10-14
Trachoo N, Mistry VV (1998) Application of ultrafiltered sweet buttermilk and sweet buttermilk powder in the manufacture of nonfat and low fat yogurts. Dairy Sci 81: 3163–3171
Vinderola CG, Reinheimer JA (1999) Culture media for the enumeration of Bifidobacterium bifidum and Lactobacillus acidophilus in the presence of yogurt bacteria. Int Dairy J 9: 497-505
Zainoldin KH, Baba AS (2009) The effect of *Hylocereus polyrhizus* and *Hylocereus undatus* on physicochemical, proteolysis and antioxidant activity in yogurt. Eng Technol 60: 361-366
Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agri Food Chem 49: 5165-5170
Zhu FM, Du B, Li J (2014) Effect of ultrafine grinding on physicochemical and antioxidant properties of dietary fiber from wine Grape pomace. Food Sci Technol Int 1:55-62

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RESEARCH ARTICLE

Assessment of market milk adulteration in Tiruchirapalli city of Tamil Nadu

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Abstract: A study was conducted to analyze the market milk samples sold in Tiruchirapalli city of Tamil Nadu for the presence of adulterants.A total of 110 milk samples, 10 from the organized brands, 50 from local vendors and 50 from tea and coffee shops were collected.The present study revealed the presence of starch in 02 milk samples(1.82%) and that to from organized brands were detected. Further, the presence of neutralizers, detergents and sodium chloride were found in 36 samples(32.73%),21 milk samples (19.09%) and 33 milk samples (30%)respectively.The skimmed milk powder was detected in all the ten organized brands. The milk samples obtained from local vendors, tea and coffee shop were negative for skimmed milk powder. The study also revealed the presence of sugar in 45 milk samples (40.91%), hydrogen peroxide in 03 samples (2.73%) and pond water/nitrate in 20 milk samples (18.18%) respectively. In addition, the study revealed that none of the samples collected were found positive for the presence of alizarin, formalin, urea, glucose/dextrose, cellulose, maltodextrin, protein and boric acid.

Keywords: Adulterants, Detection methods, Market milk, Tiruchirapalli city

Introduction

India has emerged as the largest milk producer and consumer in the world with an annual production of more than 187.7 million tones, giving a per capita availability of 394 gm per day (NDDB, 2019). India's milk production today accounts for 22.3% of the total world output and 40% of the Asia's total production.

Milk in its natural form has high food value. It supplies nutrients like proteins, fat, carbohydrates, vitamins and minerals in moderate amounts in an easily digestible form. Nutritional values and other health benefits have made milk and dairy products to be extensively consumed by large segments of the population during all stages of development and life including childhood, adolescence, pregnancy and the elderly. Adulteration can be defined as the inclusion in foods of constituents whose presence is prohibited by regulation, custom and practice or "making impure by adding inferior, alien or less desirable materials or elements"(Sonal et al. 2013).

The addition of adulterants to food to increase attractiveness and value is often referred to as "economic-adulteration".Milk may be adulterated on purpose and mostly motivated by economic greed, or accidentally during production or processing. The driving force behind most adulteration is to maximize revenues by using (partially) either a cheap ingredient as a substitute for a more expensive one, or (partially) removing the valued component in the hope that the altered product is undetected by the final user. Adulteration is an act of intentionally debasing the quality of food offered for sale either by admixture or substitution of inferior substances or by the removal of some valuable ingredients as per Food and Drug Administration (1995).

In the National Survey on Milk adulteration (2011) conducted by the Food Safety and Standards of Authority of India (FSSAI) to ascertain the quality of milk throughout the country, 68.4% samples were found to be non-conforming to Food Safety and Standards Regulations, 2011. The FSSAI latest report of October 2019 on National Milk Safety and Quality Survey which was conducted during 2018 reveals that milk in India is largely safe, even though quality issue persists. The survey found that less than 10% of milk samples had contaminants coming mainly from feeds and improper farming practices (FSSAI, 2019).

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The proposed study on adulteration of milk will bring about more awareness to the general public about the malpractices or negligence in milk production. Added to that it will establish its importance in formulating future food safety measures thus will address the health concern of the Tiruchirapalli city in particular and the state of Tamil Nadu in general with the following objectives.Collection of milk samples from organized brands, local vendors, tea and coffee shops from different areas in Tiruchirapalli city of Tamil Nadu. The collected milk samples were tested for the presence of different adulterants, *viz.,* alizarin, formalin, urea, starch, neutralizers, detergent, sodium chloride, skim milk powder, sugar, glucose, hydrogen peroxide, cellulose, maltose, ammonium sulphate, protein, boric acid, pond water / nitrate.

Materials and Methods

Collection of milk samples

Tiruchirapalli city is located at the centre of Tamil Nadu, gets most of the milk supply from the surrounding villages, private dairies and cooperative milk union.The city comprise of four zones containing 65 wards. The market milk samples were collected randomly from all the four zones. A total of 110 milk samples, 10 from the organized brands, 50 from local vendors and 50 from tea and coffee shops were collected aseptically and were transported in an icebox at a suitable temperature range (0-4°C) to the laboratory within two hours. The collected milk samples were analyzed in Veterinary University Training and Research Centre, Tiruchirapalli.

Adul-teration testing using milk kit

The kit for adulteration testing of milk was obtained from Himedia, Mumbai. The kit was designed for the detection of common seventeen milk adulterants in the milk sample (Bhanu et al. 2014).It was available in two parts, the Part A of the kit- K088A consisting of reagents for detection of 11 adulterants namely Alizarin, Formalin, Urea, Starch, Neutralizers, Detergents, Sodium chloride, Skim Milk powder, Sugars, Glucose/Dextrose and Hydrogen Peroxide. The Part B of the kit-K088B consisting of reagents for detection of 6 adulterants namely Cellulose, Maltodextrin / Maltose, Ammonium Sulphate, Proteins, Boric Acid and Pond Water/Nitrate. The kit was utilized for testing 50 samples each and was simple to carryout tests at field level and results could have been obtained rapidly. By observing colour changes we could decide the presence or absence of different adulterants (Table 1 and Fig. 1).

Results and Discussion

The market milk samples collected from organised brands, local vendors, tea and coffee shops from Tiruchirapalli city of Tamil Nadu were analysed for seventeen different adulterants and the results are presented in Table 2

Table 1 Detection of adulterants in market milk

S. No.	Adulterant	Presence	Absence
1.	Alizarin (Acidity and Heat stability)	Acidic-Reddish orange- pH 5.8 Alkaline- Reddish violet- pH 7.8	Normal milk- Red- pH 6.8
2.	Formalin	Purple / Violet colour ring	Brownish yellow coloured ring
3.	Urea	Yellow colour	Off white to slight yellow colour
4.	Starch	Blue colour	Off white to cream colour
5.	Neutralizers (Sodium bicarbonate, Sodium carbonate, Sodium hydroxide and Calcium hydroxide)	Reddish pink	Light orange
6.	Detergents (Washing powder, Shampoo)	Blue Slate Grey (Shampoo)	Grayish blue
7.	Sodium chloride	Yellow	Brick red
8.	Skim milk powder	Bluish	Greenish
9.	Sugar (sucrose)	Brick red	Pale yellow
10.	Glucose / Dextrose	Bluish green	Light green
11.	Hydrogen peroxide	Bluish grey	White to light grey
12.	Cellulose	Moss green	Yellow
13.	Maltodextrin /Maltose	Brown	Golden yellow
14.	Ammonium sulphate	Teal blue (Cons d" 0.5%) Alabaster (Cons > 0.5%)	Wheat colour
15.	Proteins	Pink	Colourless
16.	Boric acid	Reddish orange	Turmeric yellow
17.	Pond water / Nitrate	Pink magneta	White to off white

Fig. 1 Test results of milk samples for urea,starch, neutralizer, detergents, sodium chloride, sugar, cellulose and nitrate

Alizarin (acidity and heat stability)

None of the milk sample was found positive for Alizarin. Acidity is a measure of freshness and bacterial activity in milk. Among 110 milk samples tested, 71 samples had pH in normal range, 36 had acidic side and 03 in alkaline side. Most of the milk samples collected from the local milk vendors, tea and coffee shops showed acidic pH. Lack of refrigeration leads to microbial fermentation of milk sugar might be the reason for higher acidity in theses samples.

Formalin

Addition of formalin even at low concentration in milk inhibit proliferation of bacteria and help in keeping quality of milk for longer period (Bansal and Singhal, 1991). None of the milk sample was detected for presence of formalin in present study; indicate the lack of awareness of milk sellers about the use formalin for increasing keeping quality of milk. In contrast, Arora et al. (2004), Sanjeevani et al. (2011) and Abbas et al. (2013) reported the presence of formalin in milk samples at the level of 0.4 %, 12 % and 28.33% in market milk samples tested in Delhi, Nanded town in Maharashtra and Peshawar in Pakistan respectively.

Urea

Urea is usually added to milk to elevate nitrogen content and mimic a high protein concentration. Addition of water in milk leads to disappearance of foamy appearance of milk. Urea is being used to restore the milky appearance. Urea is an end product of nitrogen metabolism and a normal constituent of milk. It is usually found between 180 and 400 ppm and constitutes about 55 % of the non-protein nitrogen compounds (Paradkar et al. 2000). The cut-off concentration for urea in milk is normally set at 700 ppm (Nikoleli et al. 2010). The presence of urea in milk above this limit could cause severe health problems for humans such as indigestion, acidity, ulcers, etc. (Trivedi et al. 2009). The present study revealed that none of the milk sample was found positive for urea. Abbas et al. (2013) reported that none of the milk sample collected from Peshawar district, Pakistan was positive for urea. In contrast, detection of urea adulteration reported by Nirwal et al. (2013) in different regions of Dehradun in Uttarakhand and Deepti (2014) in Akola city of Maharashtra state.

Starch

Starch is usually added to synthetic milk to improve its appearance, mainly viscosity, to compensate the effect of water adulteration. Starch improves the value of total solids up to the level acceptable by the consumers (El-Loly et al. 2013). Among 110 milk samples tested, two samples (1.82%) were positive for starch. Ahmad (2009) collected three hundred milk samples from three different localities in Sudan and observed for adulteration with starch that 35.5 % of samples were found adulterated with starch. Abbas et al. (2013) detected 26.66 % of milk samplesadulterated with starch in Peshawar district, Pakistan. However Islam et al. (2013); Nirwal et al. (2013), El-Loly (2013), Hossain (2013) and Deepti (2014) reported that none of the milk sample was positive for starch.

Neutralizers

Neutralizers are adulterated with milk to mask the acidity and to improve the apparent quality. There were 36 samples (32.73%) out of 110 were found to be positive for neutralizers in the present study. Bhanu et al. (2014) reported presence of neutralizers in 15

Table 2 Incidences of adulteration in market milk sold in Tiruchirapalli city of Tamil Nadu

S. No.	Source of milk sample	Alizarin	Formalin	Urea	Starch	Neutralizer	Detergent	Sodium chloride	Skimmed milk powder	Sugar	Glucose/dextrose	Hydrogen peroxide	Cellulose	Maltodextrin	Ammonium sulphate	Protein	Boric acid	Pond water/ nitrate
1	Organized brands n=10	0	0	0	2(20%)	5(50%)	0	0	10 (100%)	0	0	3 (30%)	0	0	0	0	0	0
2	Local vendors n=50	0	0	0	0	10(20%)	11 (22%)	12 (24%)	0	22(44%)	0	0	0	0	0	0	0	7 (14%)
3	Tea and coffee shops n=50	0	0	0	0	21 (42%)	10 (20%)	21 (42%)	0	23(46%)	0	0	0	0	0	0	0	13 (26%)
	Number of positive samples (n=110)	0	0	0	02 (1.82%)	36 (32.73%)	21 (19.09%)	33 (30%)	10 (9.09%)	45(40.91%)	0	03(2.73%)	0	0	0	0	0	20 (18.18%)

% of market milk sold in Chennai. Deepti (2014) reported presence of neutralizers in one out of ten milk samples of different company pouch in Akola city of Maharashtra state.

Detergents

Detergents are either deliberately added to improve the appearance of milk or it may get in to milk due to improper rinsing of milk utensils after detergent wash. Out of 110 milk samples twenty-one (19.09%) was positive for detergent test in this study. As per National Survey on Milk Adulteration 2011, 8.4 % of milk samples collected throughout India was found adulterated with detergent (FSSAI Report, 2011). Similarly, Nirwal et al. (2013) reported positive results for detergent in milk quality analysis in Dehradun. In contrast, Abbas et al. (2013) reported that none of the milk sample tested was positive for detergent in Peshawar district, Pakistan.

Sodium chloride

Sodium chloride or salt are added to milk to mask higher water content. Total of 33 milk samples (30%) out of 110 tested were positive for sodium chloride. Similarly, Arora et al. (2004) observed that in all the collected milk samples from organized and unorganized sector 0.6% samples were positive for sodium chloride detection test. Abbas et al. (2013) reported 16.66 % positive results for sodium chloride test in milk samples collected from Peshawar district, Pakistan. Deepti (2014) reported very high positive results for sodium chloride in five out of ten milk samples tested in Akola city. Nirwal et al. (2013) reported 51% positive samples for sodium chloride in Dehradun.

Skim milk powder

Skim milk powder is added to milk to improve the solid not fat content of milk and/or mask the added water.In present study 10 samples (9.09%) out of 110 were positive for skim milk powder test. As per National Survey on Milk Adulteration 2011, there were 548 milk samples out of 1791 *ie.* 44.69 % positive for skim milk powder adulteration (FSSAI Report, 2011). Similarly, Deepti (2014) reported 20 % positive result for skim milk powder adulteration. Nirwal et al. (2013) reported that 58 % of milk they tested was positive for skim milk powder adulteration. But in true sense, presence of skim milk powder should not be considered as an adulterant as it is produced from milk only.

Sugar

Sugar usually added to improve the solid not fat content of milk and/or to mask the added water. There were 45 milk samples (40.91%) out of 110 collected in this study were found to be positive for added sugar. Abbas et al. (2013) reported 18.33 % sugar adulteration in milk samples collected in Peshawar district, Pakistan. Lateef et al. (2009) reported very high *i.*e. 93.33 % cane sugar adulteration in milk. Deepti (2014) detected 3 milk samples

with sugar out of 10 samples. Nirwal et al. (2013) observed 20 % milk adulteration with sugar during summer, 12 % in rainy and 3 % in winter season.

Glucose / Dextrose

Glucose or Dextrose is added to milk to enhance Solid Not Fat (SNF) portion of milk. The presence study revealed that none of the milk sample was found positive for presence of glucose or dextrose. In contrast to this, National Survey on Milk Adulteration (2011) revealed that477 milk samples out of 1791 were adulterated with glucose (Anonymous, 2011). In an assessment of adulteration in skimmed milk samples of Akola city, out of 8 milk samples there were 3 samples with glucose adulteration (Deepti, 2014). In a milk quality analysis in Dehradun conducted by Nirwal et al. (2013), 80 % of milk samples were found to be adulterated with glucose.

Hydrogen peroxide

To increase the shelf life of raw milk hydrogen peroxide is added. This unethical activity is usually adopted in summer season when the environment temperature is very high, to prevent the spoilage milk during transport (Tipu et al. 2007). The present study revealed that 03 milk samples (2.73%) out of 110 were found to be adulterated with hydrogen peroxide. There were 23.5% pasteurized milk samples and 5.58%raw milk samples foundpositive for hydrogen peroxide. (Wangala and Wafula, 2007). In contrast, none of the milk sample was found positive for hydrogen peroxide reported by Mishra et al. (1977), Patel (1979), Karpude et al. (1987), Rao et al. (2002), Abbas et al. (2013), Deepti (2014). Reason for the absence of Hydrogen peroxide adulteration was may be that it is not easily and cheaply available in the open market.

Cellulose

The present study revealed the absence of cellulose in all the 110 milk samples analyzed. Similarly, Deepti (2014) also reported that, none of the skim milk samples analyzed for adulteration from Akola city was positive for cellulose test. Absence of cellulose in any of the milk samples could be due to unawareness of cellulose among milk vendors.

Maltodextrin /Maltose

Out of 110 milk samples tested, no one was found positive for Maltodextrin / maltose. This was in agreement with Deepti (2014). The reason may be that Maltodextrin / maltose is not available cheaply in the open market.

Ammonium Sulphate

The milk vendors may add ammonium sulphate to milk in order to increases the lactometer reading by maintaining the density of milk. None of the milk sample in this experiment was adulterated with Ammonium Sulphate.

Proteins

The presence of protein milk samples vary between 3.0 and 4.1 %**.** The present study revealed that none of the milk sample was deviated from the normal range which in turn ruled out the addition of extraneous protein in the tested milk samples. In contrast, Deepti (2014) reported three skim milk samples were found above normal range of protein and none below the normal range in Akola city of Maharashtra state.

Boric acid

Out of 110 milk samples analyzed, Boric acid was not detected in any of the milk samples in this study. The result was in agreement with that of Nirwal et al. (2013) and Deepti (2014). In contrast, Abbas et al. (2013) reported 11.67 % of milk samples adulterated with Boric acid in Peshawar in Pakistan.

Pond water / Nitrate

Twenty milk samples (18.18%) out of 110 were found positive for pond water / nitrate in this study. This may be due to addition of raw water in milk samples to increase the volume by local vendors, tea and coffee shops. Deepti (2014) reported presence of one sample with pond water / nitrate out of ten samples tested in Akola city of Maharashtra state.

Conclusions

Adulteration is an act of intentionally debasing the quality of food offered for sale either by admixture or substitution of inferior substances or by the removal of some valuable ingredients. The driving force behind most adulteration is to maximize revenues by using (partially) either a cheap ingredient as a substitute for a more expensive one, or (partially) removing the valued component in the hope that the altered product is undetected by the final user. The market milk samples of Tiruchirapalli city in Tamil Nadu were analyzed for the presence of adulterants. The study revealed that nearly 41% of market milk samples were adulterated with any one of the adulterants tested. The adulteration was found to be more in milk samples collected from tea and coffee shops than those from organised brands.The present study on adulteration of market milk will bring about more awareness to the general public about the malpractices or negligence in milk production, processing and distribution to public. Added to that it will establish its importance in formulating future food safety measures which in turn address the health concern of the Tiruchirapalli city in particular and the state of Tamil Nadu in general.

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References

20th Livestock Census Report (2019) Department of Animal Husbandry and Dairying. Ministry

of Fisheries, Animal Husbandry and Dairying. Government of India. Krishi Bhawan, New Delhi, India.

Abbas MN, Khattak B, Sajid A, Ul Islam T, Jamal Q, Munir S (2013) Biochemical and bacteriological analysis of milk samples collected from district Peshawar. Int J Pharm Sci Rev Res.21: 221–26

Ahmad A (2009) Milk adulteration by adding water and starch at Khartoum State. Pak J Nutr8: 439-440

Arora S, Sharma V, Motiram D, Kishore K (2004) Status of milk adulteration in some states of North India. Ind J Dairy Sci57:65-67

Bansal A, Singhal OP (1991) Preservation of milk samples with formalin-Effect on Acidity. Ind J Dairy Sci44:573

Bhanu RV, Gunaseelan L, Pawar GR, GiriT (2014) Assessment of adulteration and microbial quality of market milk in Chennai. Indian Vet J 91: 50-51

Deepti T (2014) Assessment of adulteration found in skimmed milk samples collected from Akola City. Ind J Res 3: 171–73

El-loly MM, Mansour AA, Ahmed RO (2013) Evaluation of raw milk for common commercial additives and heat treatments. Int J Food Safe 15: 7–11

FSSAI (2019) Food Safety and Standards Authority of India Survey: Your Milk is Largely Safe. Press release on National Milk Safety and Quality Survey 2018, New Delhi, pp 1-7

Hossain M, Bellal A, Dev SR (2013) Physiochemical characteristics of various raw milk samples in a selected dairy plant of Bangladesh. Int J Engg App Sci 1: 91–96

Karpude AA, Rathi SD, Joglekar NV, Ingle UM (1987) Adulterated milk sold in Parbhani town. Asian J Dai Res 6: 83-86

Lateef M, Faraz A, Mustafa MI, Akthar P, Bashir MK (2009) Detection of adulterants and chemical composition of milk supplied to canteens of various hospitals in Faisalabad city. Pak J Zool 9: 139-142

Mishra M, Dehury M, Nayak JB (1977) Adulteration of market milk at Bhubaneswar. Ind J Vet Sci 12:378-380

National Dairy Development Board (2019) Department of Animal Husbandry, Dairying and Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India.

Nikoleli GN, Nikolelis DP, Methenitis C (2010) Construction of a simple optical sensor based on air stable lipid film with incorporated urease for the rapid detection of urea in milk. Anal Chim Acta 675: 58–63

Nirwal S, Pant R, Rai N (2013) Analysis of milk quality, adulteration and mastitis in milk samples collected from different regions of Dehradun. Int J Pharm Technol 5: 359–64

Paradkar MM, Singhal RS, Kulkarni PR (2000) An approach to the detection of synthetic milk in dairy milk: 1. Detection of urea. Int J Dairy Technol 53: 87–91

Patel RK (1979) A study on the quality of milk collected at different collection centre. Dairy guide 1: 27-29

Rao LV, Ranganadhan M, Rao VR (2002) Quality of milk and milk products marketed in Hyderabad city. Indian J Dairy Sci 55: 338

Sanjeevani B, Wadekar BR, Chavan R, Menkudale GV (2011) Survey on adulteration of the milk received from government milk scheme in Nanded town. Interlink Research Analysis 1: 4-9

Sonal G, Suman K, Neelima B and Kameshwar SYVR (2013) Screening of Adulterants in the Milk and Dairy Products of Delhi Region - A case study. Int J Pharm Technol 4: 4889-4897

Tipu MS, AltafI, Ashfaq M, Siddique (2007) Monitoring of chemical adulterants and hygienic status of market milk. Handbook published by Quality control Laboratory, University of Veterinary and Animal Science, Lahore, Pakistan, pp 7

Trivedi UB, Lakhminarayana D, KothariL, Patel G, Kapse N, Makhija K, Patel B, Panchal J (2009) Potentiometric biosensor for urea determination in milk. Sens Actuators B Chem140: 260–266

Wangala K, Wafula G (2007) Evaluation of Microbiological Quality and safety of milk marketed in Nairobi and Environs. Jomo Kenyatta University of Agriculture and Technology, Kenya

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RESEARCH ARTICLE

Effect of supplementation of β-carotene on nutrient intake, digestibility, milk yield and composition lactating crossbred cows

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Abstract: This study investigated the effect of supplementation of β-carotene on nutrient intake, digestibility, milk yield and composition lactating crossbred cows. Twenty four crossbred (Holstein × Sahiwal) cows with a close date of calving were divided into two homogeneous groups based on parity (4.62 ± 0.46) and milk production level within the current lactation (8.57 ± 0.08 kg/day of mature equivalent milk). During the lactation period, the cows of the BETA-group were fed a diet supplemented with 500 mg/cow/d of β-carotene. Compared to CONT-group (control), BETA-group (β-carotene supplementation diet) failed to increase body weight, dry matter intake and digestibility of crude protein, crude fiber and nitrogen free-extract except ether extract and intake of total digestible nutrients which increased ($p < 0.01$) in BETA-group. Overall daily milk yield, total milk yield, 3.5% fat-corrected milk, energy-corrected milk, fat yield, protein yield, lactose yield, solid not fat yield and total solids yield did not differ between two groups. No differences were observed in milk composition as fat % (3.36 ± 0.03), protein % (3.08 ± 0.04), lactose % (4.25 ± 0.03), solid not fat % (7.48 ± 0.03) and total solids % (10.80 ± 0.05) between CONT and BETA-groups. Furthermore the somatic cell count was also not different the groups. The results of the present study demonstrated that supplementation of β-carotene has no significant benefits on productive parameters of lactating crossbred cows.

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Keywords: β-carotene, Crossbred cows, Dry matter intake, Milk components, Nutrient digestibility, Somatic cell count

Introduction

For the period of early time of lactation, whenever the energy requirement of the high producers lactate animal becomes increasingly difficult to meet out, nutrition, production and health of the animal are closely correlated with each other (Bisinotto et al. 2012). Maintenance of proper milk yield with better animal health is a difficult task for dairy cattle. So, increase in efficiency of yield of milk and its quality can be fulfilled along with complete nutrition and manage mental practices during the time between beginning and later lactation period (Singh et al. 2020).

Beta-carotene (BETA) associates with the family unit of carotenoids, phytochemical pigments generally synthesized in fruits, vegetables, plants, algae, and photosynthetic bacteria (Eggersdorfer and Wyss 2018). β-carotene is the principal natural precursor of vitamin A in bovine and it is mainly provided by green grass and legume forages (FAO, 2001). It is generally known that a good concentration of β-carotene is closely correlated with cattle health and production performance (Bilen and Mecitoglu 2021). Supplemental β-carotene may enhance growth, production, rumen function and digestive function (Karadas et al. 2005).

Domestic ruminants are mainly fed on like crop residue-based diets, which are low in minerals and vitamin contents (Khanum et al. 2007). In India the cattle and buffaloes are normally fed on crop residues straws and cultivated fodder that are low density and nutritive values feeds consist of mainly lignified cellulose and hemicellulose and have been traditionally used as a main feed ingredients (around 70-75%) of ruminants diet (Kayastha et al. 2012). Ration fed to high yielding crossbred cows in India are generally deficient in minerals and vitamins A that are affecting animal productivity (Bhanderi and Garg 2012). It has been observed that the dietary level of certain vitamins and minerals necessary for body growth and production (NRC, 2001). Because based on tenth five year plan, government of India at present, the country faces a net deficit of 24.81% dry crop residues and 64.21% green fodder. The regional deficits are more essential

than the national deficit, particularly for fodder, which is no way economical to transport very long distances. So addition of mineral-vitamin in animal diets can enhance the utilization of poor quality roughages mainly and improved the production performance. The aim of present study was a novel study on Indian crossbred cows with supplemental effect of β-carotene on nutrient intake, digestibility, milk yield and composition lactating crossbred cows.

Materials and Methods

Animals and experimental diets

The study was carried out at the dairy farm, Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, on Indo Gangetic alluvial plain lands. Sahiwal crossbred (Holstein × Sahiwal) cows with a close date of calving were randomly located in individual pens to form two experimental groups: (i) Control (CONT; n = 12; parity = 4.41 ± 0.60, milk yield = 8.53 ± 0.12 kg) and (ii) beta-carotene (BETA; n = 12; parity = 4.83 ± 0.72 kg, milk yield = 8.62 ± 0.13 kg), with similar body weight between treatment groups. The cows in group BETA were orally supplemented with β-carotene (500 mg/cow/d) during the entire experimental period of 30 days. During the digestible trial consisted of 23 days for diet adaptation and 7 days for sample collection. Samples of milk were collected at an interval of 15 days until 105 days postpartum, from 2 sequential milking of morning and evening, for measurements of milk yield and milk composition. The experimental groups received a basal diet twice daily containing wheat straw (*Triticum aestivum*) *ad lib* and green lucerne (*Medicago sativa*) as a green fodder in a mixed-ration, balanced to meet their nutrient requirements (NRC; Table 1). Both groups had free access to water and shaded areas. Diets for cows in both the groups were individually offered to each cow. The only difference in BETA intake between treatments was the β-carotene supply provided to the BETA-group thereby the effect to offer or not supplemental BETA in both treatment groups was assessed.

Mineral-vitamin premix formulation

A trace mineral-vitamin premix (DSM Nutritional Products India Pvt. Ltd., Mumbai, India) was formulated as shown in (Table 2). The mineral-vitamin premix level was designed to fulfill or exceed the mineral-vitamin requirements of lactating cows according to DSM Mineral-Vitamin Supplementation Guidelines (2016) as well as NRC (2001).

Intake and apparent digestibility evaluations

Intake was evaluated daily by weighing the feed provided and the refusals recorded the next morning from each animal. Samples of the offered diets and the refusals were stored at –20°C for further analysis and further analyzed to evaluate feed intake and digestibility. In 30 days experimental period (overall 7 samplings were taken during the experiment), samples of wheat straw, green lucerne, concentrate were collected on a daily basis. Samples were dried in an exceedingly forced-air oven at 60°C for 48 h. Once dried, they were ground with a Wiley mill (2.0 mm screen) and analyzed in duplicates for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash according to the of the standard procedures of AOAC (2005). The total digestible nutrients (TDN) were estimated using the standard formula.

To determine the digestibility (g/kg) of each nutrient, the following equation was used:

$$\text{Digestibility (g/kg)} = \frac{[\text{ingested nutrient amount (g/d)} - \text{amount nutrient extracted in the feces (g/d)}]}{[\text{ingested nutrient amount (g/d)}]} \times 1000$$

Feces were collected every 24 h from each individual stall that was equipped with a metallic container with a mesh frame and sampled at 08:00 h on the last 7 days of every period (06.00 AM; 02.00 PM and 10.00 PM). A subsample of 10% was taken and stored at -20°C until analysis.

Individual body weight (BW, kg) and BW change (BWC, kg/d) were registered at the start and end of each experimental period. Dry matter intake (DMI, kg/d) was recorded on a daily basis but only data from the last 7 days were used for statistical analysis.

Measurements of milk yield and milk components

Cows were milked twice daily by hand milking and yields of milk were recorded automatically (Electric milk meters) and individual milk yields (kg/d) were recorded on a daily basis but only data from the last 15 days of each period were used for statistical analysis. Samples of milk were collected at an interval of 15 days until 105 days postpartum, from 2 sequential milking, morning and evening (4.00 A.M. and again at 4.00 P.M.). Individual milk

Table 1 Chemical composition of concentrate mixture, wheat straw and Lucerne

	Diet		
Nutrient composition	Concentrate mixture	Wheat straw	Lucerne
Crude protein	18.60	3.21	4.48
Crude fiber	9.78	36.40	5.10
Ether extract	3.45	1.12	1.04
Nitrogen free-extract	57.83	49.80	5.39
Ash	10.30	11.87	4.12

samples (500 mL) were collected on the last 15 days of each experimental period at 4.00 A.M. and again at 4.00 P.M using a volumetric milk meter. Milk samples were analyzed in duplicates by EKOMILK ultra milk analyzer (MILKANA KAM98-2A, EON TRADING, USA, made in Europe, Bulteh 2000 Ltd.) so as to work out fat, protein, lactose, solid not fat (SNF) and total solids (TS) and somatic cell count (SCC) using modified Olympus microscopic (CH20iBIMF, made in Olympus Pvt. Ltd. Nodia, India) and examined under the oil immersion lens (i NEA 100×/ 1.25 oil) at the department of dairy science and food technology laboratory.

Yields of milk corrected for 3.5% fat and for energy were calculated according to NRC (2001) as follows:

3.5% fat-corrected milk g/d = (0.4324 × milk yield g/d) + (16.218 × milk fat yield g/d)

Energy-corrected milk g/d = [(0.3246 × milk yield g/d) + (12.86 × fat yield g/d) + (7.04 × protein yield g/d)]

Feed efficiency (FE) was calculated as: milk yield (kg/d) ÷ DMI (kg/d)

Adjusted FE was calculated as: 3.5% FCM (kg/d) ÷ DMI (kg/d)

Statistical analysis

Data from the last 7 days of DMI, nutrient digestibility and fortnightly milk yield, milk components and SCC were considered for statistical analysis. Data were compared using the student's t-test procedure of the IBM SPSS statistics software package (2012). Variability in the data is expressed as the standard error of means. The probalility value $P<0.01$ was considered for significance levels.

Results and Discussion

Intake of nutrients

The BW remained similar between CONT and BETA-groups (420 ± 9.94 kg) (Table 3). A article stated that β-carotene supplementation had no effect on the average live weight or change of live weight during the trial duration (Ducker et al. 1984). Condron et al. (2014) obtained similar result of synthetic β-carotene had no effect on weight or gain compared non-supplemented group of cows during experimental period. However, no differences were observed on cattle of the supplementation vitamin A in the feed intake, average daily gain, gain-to-feed ratio (Jo et al. 2020).

During the experimental period in both the groups of CONT and BETA cows had no effect on nutrients intake of DM (13.2 ± 0.40 kg/d), CP (1.12 ± 0.01 kg/d), CF (2.50 ± 0.01 kg/d) and NFE (5.66 ± 0.03 kg/d). Adding β-carotene to BETA-group cows increased ($p < 0.01$) nutrients intake of EE (0.24 vs. 0.33 kg/d) and TDN (6.14 vs. 6.69 kg/d) when compare with CONT-group cows (Table 3), which partly agrees with what was previously reported both in lactating cows (Michal et al.1994) when animals were fed with either β-carotene enriched premix mineral-vitamin. Condron et al. (2014) reported in similar way synthetic β-carotene failed to affect daily DMI, gain:feed, or days on feed on cattle.

Table 2 Composition of the trace mineral-vitamin premix (unit/kg as-is basis)

Item	Quantity
Vitamin A (MIU)	2.000
Vitamin D_3 (MIU)	0.400
Vitamin E (g)	20.000
Biotin (g)	0.400
Niacin (g)	10.000
β-carotene (g)	10.000
Iron (g)	12.000
Copper (g)	4.000
Manganese (g)	16.000
Zinc (g)	16.000
Magnesium (g)	80.000
Cobalt (g)	0.400
Iodine (g)	0.300
Selenium (g)	0.120
Chromium (g)	0.500
Potassium (g)	5.000
Sodium (g)	6.000

Digestibility of nutrients

The apparent digestibility of nutrients in both the groups for DM, CP, CF and NFE was not different with the supplementation of β-carotene to cows (Table 3). Similar to our findings, there was no effects on apparent nutrients digestibility or utilization as observed by Dermauw et al. (2013) in zebu cattle. However animals fed with β-carotene had higher EE digestibility ($P < 0.01$) when compared to the CONT group. Our results also corroborate with that of Nogueira et al. (2017) reporting greater digestibility of EE as well as Hino et al. (1993) reporting increased utilization of fatty acids on supplementation a combination of β-carotene and α-tocopherol in the goat.

Milk yield and milk components

Data of daily milk yield 105 days of lactation of crossbred cows are presented in Fig. 1. Results revealed that daily milk yield was similar in BETA-group 5.66% (8.65 kg/d) at 15 days compared with CONT-group 5.62% (8.58 kg/d). The differences were found non-significant ($P > 0.05$) among groups being at 90 days highest with BETA-group 6.72% (10.27 kg/d) and lowest with CONT-group 6.53% (9.98 kg/d) and after 90 days lactation periods daily milk yield at 105 days slowly decrease in both groups. The overall

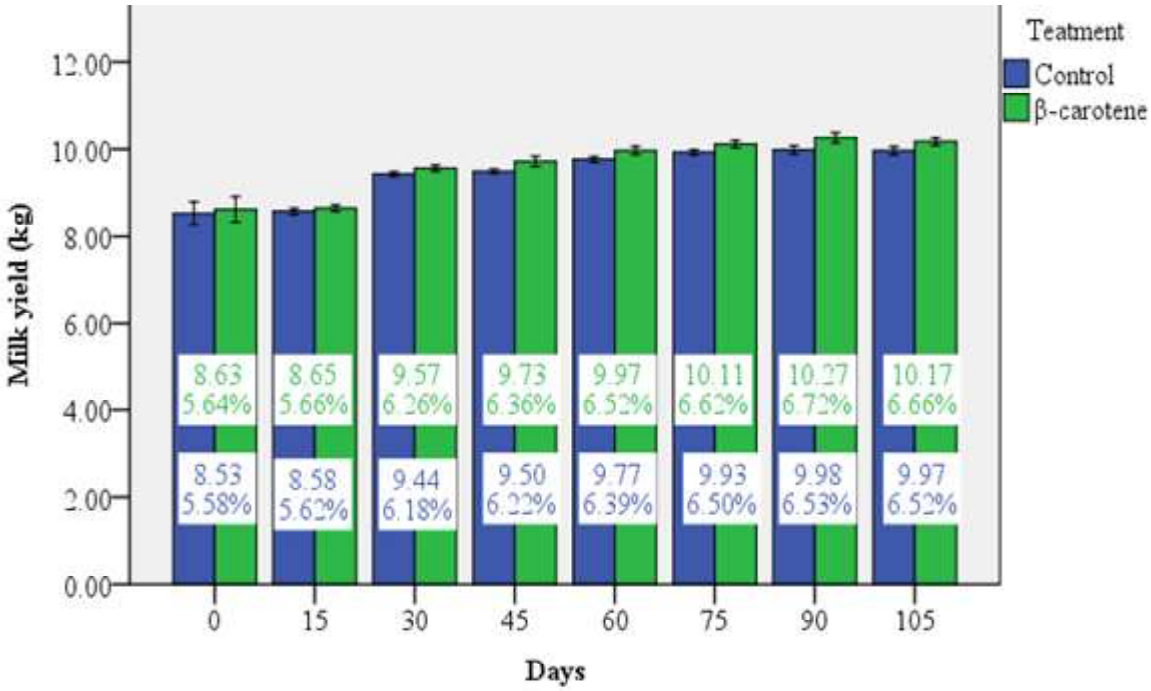

Fig. 1. Effect of supplementation of β-carotene on milk yield in crossbred cows

Table 3 Nutrient intake (kg/d) and digestibility in crossbred cows

	CONT	BETA	SEM	P-value
Average body weight (kg)	419.2[a]	421.2[a]	9.94	0.92
Intake kg/d				
Dry matter	12.9[a]	13.5[a]	0.40	0.47
Concentrate intake	5.15[a]	5.51[a]	0.17	0.32
Wheat straw intake	5.78[a]	5.96[a]	0.12	0.49
Lucerne intake	1.98[a]	2.05[a]	0.11	0.79
Crude protein	1.09[a]	1.16[a]	0.01	0.08
Crude fiber	2.47[a]	2.54[a]	0.01	0.21
Ether extract	0.24[a]	0.33[b]	0.01	<0.01
Nitrogen free-extract	5.57[a]	5.75[a]	0.03	0.07
Total digestible nutrients	6.14[a]	6.69[b]	0.09	<0.01
Digestibility coefficient (g/kg)				
Dry matter	699[a]	707[a]	8.14	0.63
Crude protein	678[a]	688[a]	11.6	0.69
Crude fiber	530[a]	552[a]	8.34	0.20
Ether extract	641[a]	796[b]	40.13	<0.01
Nitrogen free-extract	670[a]	676[a]	6.33	0.64

[a,b] Mean values for each experiment within a row with different superscript letters differ significantly ($p < 0.01$). CONT = Control group, BETA = β-carotene supplemented group, SEM = standard error of mean, TDN = %CP (dig) + %CF (dig) + %NFE (dig) + EE (dig) × 2.25

means in both groups for yields of milk, fat, protein, lactose, solid not fat and total solids were not affected (Table 4). Similar to our findings observed by De Ondarza et al. (2009) did not find changes in milk yield and its composition with supplementation of β-carotene in lactating dairy cows. The similar results finding of yields of milk and milk components yields values were increased non-significantly. However in cows the supplementation of carrot

Table 4 Milk yield and quality indicators in crossbred cows

	CONT[4]	BETA[5]	SEM	P-value
Milk yield, kg/d[105]	9464.05[a]	9637.49[a]	153.06	0.589
3.5% Fat-corrected milk[1], g/d	9215.70[a]	9498.77[a]	191.89	0.480
Energy-corrected milk[2], g/d	9158.60[a]	9500.80[a]	210.02	0.434
Feed efficiency[3]	0.73[a]	0.71[a]	0.01	0.408
Milk composition (%)				
Fat	3.32[a]	3.39[a]	0.03	0.262
Protein	3.02[a]	3.14[a]	0.04	0.178
Lactose	4.18[a]	4.31[a]	0.03	0.095
Solid not fat	7.46[a]	7.50[a]	0.03	0.516
Total solids	10.79[a]	10.82[a]	0.05	0.802
Milk composition (g/d)				
Fat	315.91[a]	328.74[a]	7.81	0.430
Protein	287.49[a]	304.66[a]	8.70	0.341
Lactose	397.35[a]	419.89[a]	9.61	0.326
Solid not fat	707.17[a]	724.03[a]	14.05	0.567
Total solids	1023.30[a]	1032.55[a]	21.79	0.840
Somatic cell count (Log 10^4 cell/mL)	12.75[a]	12.05[a]	0.17	0.470

[1]FCM (Fat corrected milk – 3.5%) g/d = (0.4324 × milk yield) + (16.218 × milk fat yield), (NRC 2001), [2]ECM (Energy corrected milk) g/d = [(0.3246 × milk yield) + (12.86 × fat yield) + (7.04 × protein yield)] (NRC 2001), [3]Feed Efficiency = Milk yield (g/d) ÷ DMI (g/d), [4]CONT = Control group, [5]BETA = β-carotene group

did not improved in the concentration of milk fat, protein, lactose and milk yield observed by Antone et al. (2015).

No differences were observed both group of cows in of 3.5% fat-corrected milk, energy-corrected milk, feed efficiency and non-significantly differ in milk composition during the experimental period (Table 4). Our results corroborate with that of De Ondarza and Engstrom (2009) the effect of supplementing β-carotene had no effect on 3.5% fat-corrected milk yield and its composition in dairy cows. In similar way no evidence was observed for positive responses to β-carotene supplementation in milk yield and milk composition between treatments in cows reported by (Kaewlamun et al. 2012; Oliveira et al. 2015). The similar result also found in goats by Gore and Lehloenya, (2020) that supplemental β-carotene did not significantly influence the average daily and total milk yield and milk composition.

Somatic cell count

The SSC was found non-significant in CONT-group (12.75 Log 10^4 cell/ml of milk) than BETA-group (12.05 Log 10^4 cell/ml of milk) those supplementation of β-carotene is show in (Table 4), which is similarly supported by Gore and Lehloenya (2020) the SCC did not differ significantly in β-carotene supplemented group than in non-supplemented group. Whereas on another hand a positively response observed by Kadyan et al. (2020) on buffaloes the effect of supplemented with β-carotene the overall milk SCC was significantly lower in β-carotene supplemented group as compared to that non-supplemented group.

Conclusions

Supplemental β-carotene significantly influence nutrients intake and digestibility of ether extract and total digestible nutrients of crossbred cows during postpartum period. Milk yield, composition and SCC did not differ significantly between β-carotene supplemented group and non-supplemented group. Further research is warranted on the supplemental effect of different levels of β-carotene on milk yield and composition and on concentration of β-carotene in blood plasma and milk at different stages of lactation in cows.

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References

Antone U, Zagorska J, Sterna V, Jemeljanovs A, Berzins A, Ikauniece D (2015) Effects of dairy cow diet supplementation with carrots on milk composition, concentration of cow blood serum carotenes, and butter oil fat-soluble antioxidative substances. Agron Res 13: 879-891

AOAC (2005) Association of Official Analytical Chemists. Official Methods of Analysis. 18th ed., AOAC: Gaithersburg, MD, USA

Bhanderi BM, Garg MR (2012) A study on reducing the incidence of sub-clinical and clinical mastitis in dairy cows by feeding a vitamins and minerals based strategic feed supplement. Indian J Dairy Sci 65:388-392

Bilen EK, Mecitoglu GY (2021) Effects of Beta-carotene Administration on Fertility in Lactating Dairy Cows. Indian J Anim Res 55: 145-149

Bisinotto RS, Greco LF, Ribeiro ES, Martinez N, Lima FS, Staples CR, Thatcher WW, Santos, JEP (2012) Influences of nutrition and metabolism on fertility of dairy cows. Anim Reprod 9: 260-272

Condron KN, Lemenager RP, Claeys MC, Lipkie TE, Schoonmaker JP (2014) Supplemental β-carotene I: Effect on plasma vitamin A, growth, performance, and carcass characteristics of feedlot cattle. J Meat Sci 98: 736-743

De Ondarza MB, Engstrom M (2009) Production and reproduction responses of dairy cows to supplemental β-carotene. Penn State Dairy Cattle Nutrition Workshop.

De Ondarza MB, Wilson JW, Engstrom M (2009) Case study effect of supplemental β-carotene on yield of milk and milk components and reproduction of dairy cows. Prof Anim Sci 25: 510-516

Dermauw V, Yisehak K, Dierenfeld ES, Du Laing G, Johan B, Wuyts B, Janssens GPJ (2013) Effects of trace element supplementation on apparent nutrient digestibility and utilisation in grass-fed zebu (*Bos indicus*) cattle. Livest Sci 155: 255-261

DSM Vitamin Supplementation Guidelines 2016 for Animal Nutrition. Available online: https://www.dsm.com/markets/anh/en_US/generic/download-registration-vitamin supplementation-guidelines-in-animalnutrition 2016.html?assetPath=/content/dam/dsm/anh/en_US/documents/Vitamin_Supp_Guidelin.pdf (accessed on 28 November 2019)

Ducker MJ, Yarrow NH, Bloomfield GA, Edwards-Weed JO (1984) The effect of β-carotene on the fertility of dairy heifers receiving maize silage. Anim Prod 39: 9-16

Eggersdorfer M, Wyss A (2018) Carotenoids in human nutrition and health. Arch Biochem Biophys 652: 18-26

FAO (2011) Rearing young ruminants on milk replacers and starter feeds. Animal Production and Health Manual Rome. 13: 19

Gore DLM, Lehloenya KC (2020) β-carotene supplementation does not improve milk yield and milk components of Saanen goats. Am J Anim Vet Sci 15: 123-128

Hino T, Andoh N, Ohgi H (1993) Effects of β-carotene and α-tocopherol on rumen bacteria in the utilization of longchain fatty acids and cellulose. J Dairy Sci 76: 600-605

Jo YH, Peng DQ, Kim WS, Kim SJ, Kim NY, Kim SH, Nejad JG, Lee JS, Lee HG (2020) The effects of vitamin A supplementation during late-stage pregnancy on longissimus dorsi muscle tissue development, birth traits, and growth performance in postnatal Korean native calves. Asian-Australas J Anim Sci 33: 742-752

Kadyan S, Gulati HK, Kumar OS, Sihag S (2020) Effect of β-carotene supplementation on milk yield and its composition in Murrah buffaloes. Haryana Vet 59: 206-209

Kaewlamun W, Okouyi M, Humblot, P, Remy D, Techakumphu M, Duvaux-ponter C, Ponter AA (2012) Effects of a dietary supplement of β-carotene given during the dry period on milk production and circulating hormones and metabolites in dairy cows. Revue Med Vet 163: 235-241.

Karadas F, Pappas AC, Surai PF, Speake BK (2005) Embryonic development within carotenoid-enriched eggs influences the post-hatch carotenoid status of the chicken. Comp Biochem Physio. Part B, Biochemistry and Molecular Biology 14: 244-251

Kayastha TB, Dutta S, Kayastha, RB Deka RS (2012) Growth performance and nutrient utilization of growing calves with urea treated wheat straw based ration. Indian J Dairy Sci 65: 435-438

Khanum SA, Yaqoob T, Sadaf S, Hussain M, Jabbar MA, Hussain HN, Kausar R, Rehman S (2007) Nutritional evaluation of various feedstuffs for livestock production using in vitro gas method. Pakistan Vet J 27: 129-133

Michal JJ, Heirman LR, Wong TS, Chew BP (1994) Modulatory effects of dietary beta-carotene on blood and mammary leukocyte function in periparturient dairy cows. J Dairy Sci 77: 1408-1421

Nogueira RGS, Junior FP, Pereira ASC, Rodrigues, PHM (2019) Nutrient digestibility and changes in feeding behavior of cattle fed cottonseed and vitamin E. Sci Agric 76: 112-122

NRC (2001) Nutrient Requirements of Dairy Cattle: Seventh Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/9825

Oliveira RC, Guerreiro BM, Morais Junior NN, Araujo RL, Pereira RAN, Pereir MN (2015) Supplementation of prepartum dairy cows with β-carotene. J Dairy Sci 98: 6304-6314

Singh AK, Bhakat C, Kumari T, Mandal DK, Chatterjee A, Karunakaran M, Dutta TK (2020) Influence of pre and postpartum alpha-tocopherol supplementation on milk yield, milk quality, and udder health of Jersey crossbred cows at tropical lower Gangetic region. Vet World 13: 2006-2011

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RESEARCH ARTICLE

Estimation and comparison of different lactation persistency methods in Tharparkar cattle

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Abstract: The present study was undertaken to evaluate the various measures of lactation persistency and to estimate effect of various non-genetic factors affecting the persistency of milk production and comparison of different persistency indices using standard error as percentage of mean in Tharparkar cattle. A total of 322 lactation records of 138 Tharparkar cattle were collected from history cum pedigree sheet maintained at Animal Genetics and Breeding Division of National Dairy Research Institute, Karnal from 1996 to 2018 for the estimation of lactation persistency. Lactation persistency was estimated by using Ratio method (P1), Prasad method (P2), Solkner and Fuchs variation method in 200 days (P3) and 305 days (P4). The least squares means for persistency of milk yield were 149.6 ± 3.07, 0.53 ± 0.007, 1.83 ± 0.05 and 2.28 ± 0.07 in P1, P2, P3 and P4 respectively in Tharparkar cattle. Based on standard error as percentage of mean, the best method was Prasad method (1.1%). The highest values of least squares means were found for first calvers in P1 but in P3 and P4 fourth calvers were more persistent. The cows calved during rainy season were more persistent as estimated by P1 and P2 but based on P3 and P4 winter calvers were more persistent. There was a highly significant (P<0.05) positive correlation of total milk yield with all the method of persistency indices.

Keywords: Non-genetic factors, Lactation persistency, Standard error as percentage of mean, Tharparkar cattle

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Introduction

The important norm for productivity of any lactating cow is maintenance of peak yield for a longer period. Milk yield in cows steadily increases for first 45-90 days after parturition, remains stable for a few weeks and then declines. This trend of milk yield is called lactation curve (Rakes et al. 1963). Lactation curve passes through different phases, one among them is persistency. Persistency may be defined as number of days during which the level of constant yield is maintained (Grossman et al. 1999). Highly persistent cows are relatively higher milk producers and have longer productive life (Narain and Dutta 1981; Malhotra et al.1984; Ramchandraiah et al.1990)

The effect of different non-genetic factors like, season, lactation order and period of calving on persistency was estimated. Different measures of persistency indices had shown wide range of heritability estimates from low to medium with high standard error. The positive correlation of production traits with measures of persistency indices showed association and usefulness of persistency measure. So, high degree of persistency during the first lactation is desirable and can be used as one of the selection criteria for dairy animals (Narain and Dutta 1981)

However, till date no attempt has been made to study the measures of persistency indices and the environmental factors influencing the indices in Tharparkar cattle. Therefore, the present work is an attempt to study these phases of persistency of milk production in Tharparkar cattle.

Materials and Methods

The relevant data for this study was collected from 322 lactation records (305-day/less) irrespective of the lactation order of 138 Tharparkar cows from history-cum-pedigree sheets maintained at Livestock record unit, Animal Genetics and Breeding Division of National Dairy Research Institute, Karnal from 1996 to 2018 (22 years).The normal lactation was considered as the period of milk production by a cow for at least 100 days and more than 500 kg milk yield. Lactation order was classified as first, second, third and fourth and later lactations. Each year was classified into four seasons namely winter (December-March), summer (April-June),

rainy (July-September) and autumn (October-November) based on agro-climatic conditions. In order to examine the effect of year of calving on persistency of lactation, the data of 22 years from 1996 to 2018 was divided into four periods (1996-2002, 2003-2008, 2009-2013, 2014-2018).

Estimation of Persistency Indices:

Different methods of measurement of persistency used in this study are as follows:

Rao and Sundaresan (1982) method:

$$P = \frac{\text{Lactation milk yield}}{\text{Peak yield}}$$

Prasad method (1999):

A new measure of persistency estimation in which area under lactation curve during post peak period was taken into consideration:

$$P = \frac{\text{Milk production during post peak period}}{\text{Peak yield} \quad \mathbf{X} \quad \text{post peak period}}$$

$$P = \frac{\text{Average milk production during post peak period}}{\text{Peak yield}}$$

Solkner and Fuchs method (1987):

SD2 = Standard deviation of test-day milk yields (200 days)

SD3 = Standard deviation of test-day milk yields (300 days)

The data used in the present study had unequal sub-class frequencies. Disproportionate sub-class frequencies caused the different classes of effects to be non-orthogonal.

The data was classified into different lactation order, seasons, period of calving and were adjusted using Least-squares analysis, as described by Harvey (1990) model:

$$Y_{ijk} = \mu + P_i + S_j + PC_k + e_{ijk}$$

Where,

Y_{ijk} = Observation on the n^{th} individual born in i^{th} parity, j^{th} season, k^{th} period of calving

μ = Overall population mean

P_i = Effect of i^{th} parity

S_j = Effect of j^{th} season

PC_k = Effect of k^{th} period of calving

e_{ijk} = Random error, assumed to be normally and independently distributed with mean zero and constant variance i.e. NID $(0, \sigma^2_e)$

DMRT modified by Kramer (1957) was used for testing differences among least-squares means. The correlation of persistency indices with peak yield and lactation milk production was estimated by standard correlation formula using SPSS (2001) statistical package for social science.

Results and Discussion

Estimation of persistency indices by different methods:

The persistency indices were estimated by four different methods viz. P1: Ratio method (Rao and Sundaresan, 1982), P2: Prasad method (Prasad et al. 1999), P3: Solkner and Fuchs variation method in 200 days and P4: Solkner and Fuchs variation method in 305 days (Solkner and Fuchs, 1987). The overall least squares means for persistency of milk yield was 149.6 ± 3.07, 0.53± 0.007, 1.83 ± 0.05 and 2.28± 0.07 in P1, P2, P3 and P4 respectively in Tharparkar cattle (Table 3).

The overall least squares mean estimate of persistency by Ratio method of Rao and Sundaresan (1982) was 174.79 and that of Bhutia and Pandey (1989) was 185.14 in Holstein-Friesian crosses. Kumar and Singh (2004, 2006) assessed average persistency as 215.09 ± 2.88 and 207.88 ± 2.18 in Karan Fries cows. These findings were higher than our estimate in Tharparkar cattle. In Sahiwal cattle low lactation persistency of 87.86 ± 1.61 reported by Sachan et al. (2020) and 194 ± 1.10 was reported by Kaushal et al. (2016).

When compared Prasad method the least square mean of 0.53 ± 0.007 in Tharparkar cattle was lesser than the estimates found in Karan Fries cattle (0.68 ± 0.00) by Kumar and Singh (2006) and in Crossbred HF (0.621 ± 0.09) by Sharma et al. (2018).

The persistency estimates using Solkner and Fuchs (200 days and 305 days) methods were 1.83±0.05 and 2.28±0.07 respectively in Tharparkar cattle However, using Solkner and Fuchs (1987) persistency estimate were 2.43±0.005 (200 days) and 3.08±0.005 (305 days) respectively in Simmental cattle.

Atashi et al. (2006) estimated by solkner and Fuchs 305 days method in Holstein Friesian cattle (4.120±0.0079) which was higher than Tharparkar cattle. Kaushal et al. (2016) in NDRI, Karnal estimated P3 and P4 as 2.29 ± 0.04 and 2.20 ± 0.03 in Sahiwal cattle.

The effect of various non-genetic factors calculated by least squares analysis of variance using mixed model (model-1), by taking parity, season and period of calving as the fixed effect. The mean sums of squares of each fixed effect are shown in Table 2. The effect of lactation number (parity) was highly significant (P≤0.01) in P1, P3 and P4 measures of persistency but non-significant in P2 (Table 2). The highest values of least squares

means (179.41 ± 5.9) were found for first calvers in P1. But in P3 and P4 fourth calvers were more persistent (2.15 ± 0.11) and (2.74 ± 0.22) (Table 3). In P1 first two parities were significantly different from the remaining parities and there was no statistical difference from 3rd parity onwards. But in P3 and P4 first three parities were significantly different and fourth parity onwards no significant difference was observed in persistency.

Significant effect of parity was observed while comparing effect of parity on lactation persistency using different methods in Tharparkar cattle. In different lactations, persistency was increasing from 1st lactation to 4th lactation except in Ratio method. Gill (1971) and Singh and Shukla (1985) noticed that in Hariana and Gir cattle the parity had highly significant effect on persistency. Zurwan et al. (2017) in Sahiwal, Kumar and Singh (2006) in Karan Fries and Fadlelmoula et al. (2007) in crossbred HF observed that persistency decreased by 8-10% from first to second and third lactation. Persistency of milk yield were continuously increasing from 1st to 4th parity was also reported by Singh and Gopal (1982) in Rathi, Shahare et al. (1988) in HF × Hariana, Jersey × Sahiwal, Jersey × Gaolao and HF × Gaolao and Patond et al. (2014) in Jersey cattle and Garudkar et al. (2018) in Phule Triveni cattle. Several workers affirmed that first calvers had more persistency than the calvers of subsequent lactations in different cattle breeds and crossbreds (Kumar and Singh 2006; Rao and Sundaresan 1982). In Holstein Friesian cattle the lactation length of a first parity cow was, on average, 7.8, 8.6, and 8.4 days shorter than that of second, third, and fourth parity cows, respectively (William et al. 2021)

The season of calving had highly significant effect (P≤0.01) on P3 and P4 and significant effect (P≤0.05) on P1 but not significant in P2 method (Table 2). The cows calved in the rainy season were more persistent in P1 estimate (162.6 ± 6.9) and least persistent (140.2 ± 4.12) in winter season calvers (Table 3). The winter and rainy seasons were significantly different from other seasons. But when compared with Solkner and Fuchs method in P3 and P4 winter calvers had more persistency 2.21 ± 0.06 and 2.85 ± 0.11 respectively.

Season is one of the key factors affecting persistency. The present results on significant effect of season of calving were in agreement with the outcomes of Rao and Sundaresan (1982), Solkner and Fuchs (1987), Kumar and Singh (2004; 2006), Kaushal et al. (2016) in different breeds of cattle. According to Ratio method rainy calvers had more persistency than other seasonal calvers. This may be due to continuous availability of green fodder and less stress due to favourable climate conditions and least persistency in winter season may be due to unfavourable climatic condition, non-availability of green fodder and lack of nutrients. Rao and Sundaresan (1982), Prasad et al. (1999) and Kumar and Singh (2004; 2006) also testified that rainy calvers were more persistent and winter calvers were less persistent. Hence, it is inferred that variation in season can cause stress in cattle and leads to reduction in persistency. Solkner and Fuchs (1987) and Sharma et al. (2018) reported high value of persistency measure in winter season for Simmental cattle and Crossbred HF cattle respectively. The scale of measurement in Solkner and Fuchs method is different in such a way that they are measuring the deviation of milk yield from the means. Singh et al. (1965) reported that Hariana cattle which calved in the summer season had more persistency. So based on the breed and their adaptability to different environmental conditions will change therefore they show variation in lactation persistency also. Some workers reported the non-significant effect of season of calving in PhuleTriveni cattle by Garudkar (2018); Jersey breed by Patond et al. (2014); Gir by Shingare et al. (2015) and Jersey x Hariana by Koley et al. (1979). Williams et al. (2021) reported that, the lactation length of cow calving in February, March, or April was, on average, 4.2, 12.7, and 21.9 days respectively shorter, relative to cows calving in January. By optimised reproductive management and breeding programmes lead to the improved the reproductive performance of the herd thereby increased the proportion of animals calving in earlier months, which would be advantageous to lengthen

Table 1 Standard error as percentage of mean for different measures of persistency indices

Persistency indices	Measures of persistency	SE as % Mean
Ratio method	P1	1.8%
Prasad method	P2	1.1%
Solkner and Fuchs variation (200 days)	P3	2.1%
Solkner and Fuchs variation (305 days)	P4	3.5%

Table 2 Mean sum of squares of different measures of persistency indices

Source of variation	D.F	P1	P2	P3	P4
Parity	4	19960.81**	0.0027	7.37**	5.95**
Season	3	7433.18*	0.0087	5.78**	5.90**
Period of calving	5	10401.27**	0.049*	1.27*	1.14
Error	11	2206.51	0.0132	0.1184	0.5492

(** = P < 0.01 and * = P < 0.05)

Table 3 Parity, Season and period of calving wise least square means and their standard errors for different measures of persistency indices

Class	No. of observation	P1	P2	P3	P4
Mean	322	149.6 ± 3.07	0.53 ± 0.007	1.83 ± 0.05	2.28 ± 0.07
		Parity			
1	72	179.41^{d} ± 5.9	0.53 ± 0.01	1.22^{a} ± 0 .08	1.42^{a} ± 0.14
2	77	152.45^{c} ± 5.5	0 .53 ± 0.01	1.74^{b} ± 0.08	2.16^{b} ± 0.15
3	46	143.84^{b} ± 6.9	0.52 ± 0 .01	1.93^{c} ± 0.11	2.43^{c} ± 0.19
4	45	132.1^{a} ± 7.23	0.54 ± 0.01	2.15^{d} ± 0.11	2.74^{d} ± 0.22
5 to 8	81	140.26^{b} ± 5.8	0.54 ± 0 .01	2.11^{d} ± 0.09	2.68^{d} ± 0.15
		Season			
Winter	139	140.2^{a} ± 4.12	0.54 ± 0.01	2.21^{a} ± 0.06	2.85^{a} ± 0.11
Summer	95	153.3^{b} ± 4.8	0.53 ± 0.01	1.74^{b} ± 0.07	2.26^{b} ± 0.12
Rainy	47	162.6^{c} ± 6.9	0.54 ± 0.01	1.58^{c} ± 0.11	1.75^{c} ± 0 .18
Autumn	40	142.26^{a} ± 7.5	0.51 ± 0.01	1.78^{b} ± 0.11	2.27 ± 0.20
		Period of calving			
1996-2002	94	164.6^{c} ± 5.2	0.53^{b} ± 0.01	1.73^{a} ± 0.07	2.17 ± 0.12
2003-2008	74	145.8^{b} ± 5.7	0.51^{a} ± 0.01	1.85^{b} ± 0.09	2.36 ± 0.14
2009-2013	62	135.3^{a} ± 6.2	0.53^{b} ± 0.01	2.04^{c} ± 0.10	2.56 ± 0.19
2014-2018	91	152.5^{b} ± 5.3	0.57^{c} ± 0.01	1.70^{a} ± 0.08	2.04 ± 0.15

Superscripts are based on the significant difference between the levels for each non-genetic factors which are significant at $P < 0.01$ or $P < 0.05$

Table 4 Phenotypic correlation of lactation persistency with production traits

Production traits	P1	P2	P3	P4
TMY	0.641**	0.28**	0.16**	0.49**
LL	0.741**	0.23**	-0.11	-0.17*
PY	0.035	-0.22**	0.75**	0.87**

(** = $P < 0.01$ and * = $P < 0.05$)

lactations and milk yield. These achievements, the effect of function of seasonal-calving systems where all cows tend to be dried off in early winter thereby escape from winter stress. (Berry et al. 2014; Ma et al. 2019).

The effect of period of calving was highly significant ($P \leq 0.01$) in P1 and significant ($P \leq 0.05$) in P2 and P3 but not in P4 (Table 2). In P1 method, 1996-2002 period had shown more persistency while in P2 method, 2014-2018 and in P3 method, 2009-2013 period had shown more persistency. On comparison the lowest persistency during 2009-13, 2003-2008, 2014-18 periods was observed in P1, P2 and P3 respectively.

The present findings of significance of period of calving in Tharparkar cattle were in support with that of Tekerli et al. (2000), Rekik et al. (2003), Kumar and Singh (2004; 2006) and Fadlelmoula et al. (2007) in different crossbred and exotic cattle and Kaushal et al. (2016) in Sahiwal cattle. No regular trend in different measures of persistency was observed over different periods. There was a change in climate, managemental practices and genetic variations that caused difference in performance from 1996 to 2018. The effect of each non-genetic factor on different persistency estimation methods is diverse. So, different level of significance was obtained in each period. Period of calving had no significant effect on persistency of lactation was reported by Koley et al. (1979) in Jersey x Hariana crossbred; Gupta and Johar (1982) in Tharparkar; Zaman et al. (1994) in Jersey cattle and Patond et al. (2014) in Jersey cross.

Comparison of different methods of estimation of persistency

Different indices for estimation of persistency were compared on the basis of standard error (SE) expressed as percent of population mean. The lower the SE expressed as percent of population mean, the higher would be efficiency of the persistency parameters (Kaushal et al. 2016). When compared all the indices Prasad method had the least SE as percentage of mean therefore, it was the best of persistency index based on the data (Table 1). The ratio method (P1and P2) has the least SE as percentage of mean when compared with variation method.

Correlation of persistency indices with production traits

There was positive significant correlation ($P \leq 0.01$) of total milk yield (TMY) with P1, P2, P3 and P4. The correlation between

lactation length (LL) and P1, P2 were highly significant (P≤0.01) and correlation between peak yield (PY) and P2, P3 and P4 were also highly significant (P≤ 0.01). There was significant negative correlation between peak yield and Prasad method of estimation and also significant negative correlation between Solkner and Fuchs 305 day's method with lactation length (Table 4). In Tharparkar cattle, highly significant positive correlation of lactation persistency with total milk yield (TMY) was found, similar results were reported by Saxena and Kumar (1960), Shewta (2018) and Sharma and Bhatnagar (1972) in Sahiwal cattle as 0.79, 0.5 and 0.56 ± 0.4 respectively and Gill et al. (1971) in Hariana cattle (0.36 ± 0.02) and Shingare et al. (2015) found in Deoni cattle (0.60 ± 0.47). In Tharparkar cattle negative correlation of peak yield with lactation persistency was found for Prasad method and similar results were reported by Gill et al. (1971) and Singh et al. (1965) in Hariana cattle (-0.007 and -0.16 respectively). Positive correlation of peak yield with lactation persistency was stated by Torshiz (2016), Gupta and Johar (1982) and Sharma and Bhatnagar (1972) in different indigenous and crossbred cattle. In most of the reports lactation length have positive correlation with lactation persistency similar to Tharparkar cattle but persistency estimation using Solkner and Fuchs (200 and 305 days) method had negative correlation with lactation length.

Conclusions

The present investigation was conducted to study the persistency of milk production and comparison of different estimation methods based on least standard error as percentage of mean in Tharparkar cattle and effect of different non-genetic factors affecting the persistency. The overall least squares means for persistency of milk yield was 149.6 ± 3.07, 0.53 ± 0.007, 1.83 ± 0.05 and 2.28 ± 0.07 in P1, P2, P3 and P4 respectively in Tharparkar cattle. When compared all the indices, Prasad method had the least SE as percentage of mean therefore it was the best of persistency index based on the data. All the non-genetic factors were significant in Ratio and Solkner and Fuchs (200 days) methods of estimation but only period of calving was significant in Prasad method. In Solkner and Fuchs 305 days' variation method, all the non-genetic factors except period of calving was significant. In Tharparkar cattle, first calvers had more persistency than continuing parity and high positive correlation of persistency methods with total milk yield shows the significance of persistency. So lactation persistency may be used as selection criteria for the selection of best producing animals.

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Compliance with ethical standards

The research plan was approved by NDRI institutional Animal Ethics committee as per CPCSEA rules.

Reference

Atashi H, Shahrbabak M, Abdolmohammadi A (2006) Study of some suggested measures of milk yield, persistency and their relationships. Int J Agric Biol 8: 387-390

Berry DP, Wall E, Pryce JE (2014) Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8:105-21

Bhutia ST, Pandey RS (1989) A note on comparative study of persistency and its association ship with peak and total yield in dairy cattle. Indian J Dairy Sci 42:1

Fadlelmoula AA, Yousif IA, Abu Nikhaila AM (2007) Lactation Curve and Persistency of Crossbred Dairy Cows in the Sudan. J Appl Sci Res 3: 1127-1133

Garudkar SR, Pachpute ST and Deokar DK (2018) Studies on Persistency of Milk Yield and Its Association with Production Traits in Phule Triveni Synthetic Cow. Int J Curr Microbiol App Sci 6: 1585-1589

Gill GS, Balaine DS, Acharya RM (1970) Persistency and peak yield in Hariana cattle. 1. Effect of environmental and physiological factors. Indian J Anim Sci 40: 563-568.

Gill GS, Balaine S, Acharya RM (1971) Persistency and peak yield in Hariana cattle. 2. Phenotypic and genetic parameters. Indian J Anim Sci

Grossman M, Hartz SM, Koop's WJ (1999) Persistency of lactation yield: a novel approach. Indian J Dairy Sci 82: 2192-2197

Gupta LR, Johar KS (1982) Genetic and non-genetic factors affecting persistency of first lactation in Tharparkar cattle. Indian J Dairy Sci 35: 99-101

Harvey WR (1990) User's Guide for LSMLMW, Mixed Model Least-Squares and Maximum Likelihood Computer Programme. Ohio State University, Columbus, Mimeo

Kaushal S, Gandhi RS, Singh A, Chaudhari MV, Prakash V and Gupta A (2016) Efficiency of various measures of persistency of milk yield in Sahiwal cattle. Indian J Anim Res 50: 268-270

Koley Chaudhary G, Mitra DK (1979) Persistency of lactation yield in Jersey, Hariana crossbred cows. Indian J Dairy Sci 32: 302-305

Kramer CY (1957) Extension of multiple range test to group correlated adjusted means. Biometrics 13: 13-18

Kumar A, Singh A (2004) Genetic evaluation of peak yield and its relationship with persistency and lactation milk production in Karan Fries cattle. Indian J Dairy Sci 57: 416-420

Kumar A, Singh A (2006) Genetic and Environmental factors influencing persistency of milk production in Karan Fries cattle. Indian J Anim Res 40: 95-100

Ma L, Cole JB, Da YA, Van Raden PM (2019) Symposium review: genetics, genome-wide association study, and genetic improvement of dairy fertility traits. J Dairy Sci 102:3735-3743

Malhotra PK, Dutta OP and Malhotra JC (1984) Persistency of milk yield of Murrah buffaloes registered in herd book under village conditions. Indian J Anim Sci 54**:** 145-148

Narain P, Dutta OP (1981) Inheritance of part-lactation and estimation of persistency of milk yield in Sahiwal cattle. Indian J Anim Gen Breed 3: 4-10

Patond MN, Khutal BB, Pachpute ST, Ramod SS (2014) Effect of non-genetic factors on persistency of milk yield in Jersey cattle. Vet Sci Res J. 5: 1–4

Prasad S, Singh R, Bisht GS (1999) Measure of persistency and its relationship with peak yield and lactation milk yield. Indian J Dairy Sci 52: 308-314

Rakes JM, Stallcup OT, Gifford W (1963) Persistency and the lactation curve of dairy cows, Agricultural Experiment Station, Division of Agriculture, University of Arkansas, Fayetteville, Ark

Ramchandraiah K, Kumar KS, Srocmanoaryana O (1990) A study of lactation persistency in relation to certain economic traits in purebred Jersey cows. Indian J Dairy Sci 43**:** 270-273

Rao MK, Sundaresan D (1982) Factors affecting the shape of lactation curve in Friesian x Sahiwal crossbred cows. Indian J Dairy Sci 35: 160-167

Rekik B, Ben Gara A, Ben Hamouda M, Hammami H (2003) Fitting lactation curves of dairy cattle in different types of herds in Tunisia. Livest Prod Sci 83: 309-315

Sachan S, Gupta ID, Verma A, Gupta AK, Vineeth MR and Kumar A (2020) Association of lactation persistency with genetic variants of bovine growth hormone gene in Indian Sahiwal cows Indian J Anim Sci 90: 739-743

Saxena PN, Kumar S (1960) Persistency of milk yield in Sahiwal cows. Indian J Dairy Sci 13: 45-60

Shahare RB, Ali SZ, Tingare SB (1988) Genetic and non-genetic factors affecting persistency of lactation in crossbred cows. Indian J Anim Prodn Mgmt 4: 36

Sharma N, Narang R, Kashyap N, Kumari S, Kaur S, Ratwan P (2018) Genetic analysis of persistency in HF crossbreed cattle at an organised farm of northern India. Trop Anim Health Prod 50:1219-1225

Sharma RC (1972) Inheritance of persistency index and relationship of production traits to persistency index in Sahiwal, Red Sindhi and Brown Swiss crossbred cows. M.Sc. dissertation submitted to Panjab University, Chandigarh, India.

Shingare VM, Chauhan DS, Bhise BR, Ghosh N (2015) Estimates of Genetic Parameters and Trends of Lactation Performance Traits of Deoni Cattle. Theriogenol Insight-An Int J Reprod Anim 5: 69-79

Singh J, Shukla, KP (1985) Factors affecting persistency of milk production in Gir cattle. Indian Vet J 62: 888-894

Singh RP, Gopal R (1982) Persistency and peak yield of cattle in a rural area. Ind J Anim Sci 52: 487-489

Singh SB, Dutt M, Desai RN (1965) Persistency of milk yield in Hariana cattle. Ind J Vet Sci 35: 249-257

Solkner J, Fuchs W (1987) A comparison of different measures of persistency with special reference to variation of test-day milk yield. Livest Prod Sci 16: 305- 319

Tekerli M, Akinci Z, Dogan I, Akcan A (2000) Factors affecting the shape or lactation curves of Holstein cows from the Balikesir province of Turkey. J Dairy Sci 83: 1381-1386

Torshiz ME (2016) Effects of season and age at first calving on genetic and phenotypic characteristics of lactation curve parameters in Holstein cows. J Anim Sci Technol 58: 8

Williams M, Murphy CP, Sleator RD, Ring SC, Berry DP (2021) Genetic and nongenetic factors associated with lactation length in seasonal-calving, pasture-based dairy cows. J Dairy Sci 104:561-574

Zaman G, Das D, Roy TC (1994) Persistency of first lactation milk yield in a Jersey herd of Assam. J Assam Vet Council 4: 18-21

Zurwan A, Moaeen ud Din M, Bilal G, Khan MS (2017) Estimation of genetic parameters for persistency of lactation in Sahiwal dairy cattle. Pak J Zool 49: 877 882

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RESEARCH ARTICLE

Associations of udder biometry with subclinical mastitis in Gir crossbred cows

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Abstract: The investigation was undertaken on 110 Gir crossbred cows of different order of lactation and stage of lactation maintained at Research cum Development Project on Cattle, MPKV, Rahuri. The overall least squares means of udder length, udder width, udder depth and udder circumference were 65.23 ± 1.45, 63.24 ± 1.45, 19.03 ± 0.51 and 86.49 ± 1.50 cm respectively. The least squares analysis of variance showed significant (P<0.05) effect due to order of lactation on udder length in crossbred cows. Also, order of lactation had significant (P<0.01) effect on udder width and udder circumference. However, stage of lactation exerted non-significant influence on all udder measurement traits. In crossbred cows, udder length had positive and significant (P<0.01) correlation with udder width, udder circumference and udder depth. The correlation of udder width with udder circumference and udder depth was positive and highly significant (P<0.01). There was positive and significant (P<0.01) correlation of udder circumference with udder depth. The frequencies of trough, round, goaty and pendulous type udder in crossbred cows were 63.64%, 8.18%, 11.82% and 16.36% respectively. Among 110 cows, 48 (43.64%) cows were found positive for subclinical mastitis. The higher occurrence of subclinical mastitis was observed in cows having pendulous udder (66.66%) followed by goaty (53.84%), round (44.44%) and trough (35.71%) shape udder. Maximum number of positive subclinical mastitis cases were found in larger udder length (>75 cm), udder width (>75 cm), udder depth (>25 cm) and udder circumference (>95 cm). Highest occurrence (78.26%) of subclinical mastitis was found in cows of sixth and above lactation. Maximum occurrence of subclinical mastitis was noted in cows which were in late (46.15%) stage of lactation followed by early (40%) and mid (37%) stage of lactation. The traits udder shape, udder length and udder depth had positive and significant association with incidence of subclinical mastitis in crossbred cows.

Keywords: Crossbred, Mastitis, Pendulous, Udder

Introduction

Mastitis is one of the most prevailing diseases of high yielding dairy animals. It has been recognized for more than a century, and still continues to be a major cause of economic loss. Mastitis is a complex disease resulting from the interaction of infectious agents and poor managemental practices in dairy animals. A dairy herd without mastitis is virtually impossible under modern intensive farming conditions. Subclinical mastitis (SCM) is more common than clinical mastitis, but often goes undetected and given less importance than clinical mastitis. Physical characteristics of udder and teats are important traits associated with incidence of subclinical mastitis (George et al. 2007). Udder is the first site judgment of local brokers or animal husbandry men in our country for judging the milking ability of animals. Therefore, it is more important to have knowledge of morphology of udder and teats and its relation with the mastitis within this subclinical mastitis. Mastitis is an economically important disease of high yielding crossbred cows. It can occur in clinical and subclinical form. Clinical mastitis is readily apparent and easily detected by abnormalities in milk or the udder or the occurrence of secondary clinical signs. The prevalence of subclinical mastitis in dairy herds is often surprising to producers, moreover, subclinically infected udder quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk et al. 2003). The invisible changes in subclinical mastitis can be recognized by California Mastitis Test (CMT). Hence the investigation was undertaken to record the udder measurements, categories various shapes of udder and estimate association of various udder traits with sub-clinical mastitis of crossbred cows.

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Materials and Methods

The observations for study were recorded for 110 Jersey X Gir halfbred (JG), HF X Gir halfbred (FG) and Phule Triveni (FJG) crossbred cows maintained at Research cum Development Project on Cattle, Mahatma Phule Krishi Vidyapeeth, Rahuri, District Ahmednagar (M.S.). The udder measurement traits were recorded on the basis of order of lactation and stage of lactation of crossbred cows. The observations of crossbred cows recorded were biometry of udder, shape of udder and status of subclinical mastitis. Udder length, width, depth and circumference were measured one to two hours before the evening milking after securing the animals properly in a standing position on a leveled pucca floor for the accuracy. All the measurements were recorded in centimeters. Shape of udder was determined through visual appraisal method adopted by Cerkascenko (1958) and accordingly categorized into different types, viz. trough, round, goaty and pendulous. California Mastitis Test developed by Pyorala (2003) was performed directly in the cowshed.

Statistical analysis

Statistical analysis of data was done by using standard procedures viz. mean, standard error, coefficient of variations, frequency distribution, analysis of variance and correlation coefficient. In order to overcome non-orthogonality of data resulting from unequal and disproportionate subclass frequencies, the least squares method as suggested by Harvey (1990) was used for analysis. Duncan's multiple range test as modified by Kramer (1957) was used to make pair wise comparison among the least squares means with the use of inverse elements and root mean squares for error.

The correlation coefficients of various traits were computed according to the formula given by Rao (1985).

$$r = \frac{\sum Xi\,Yi - \frac{(\sum Xi)(\sum Yi)}{N}}{\sqrt{\left(\sum Xi^2 - \frac{(\sum Xi)^2}{N}\right)\left(\sum Xi^2 - \frac{(\sum Yi)^2}{N}\right)}}$$

Where,

Xi and Yi are two variables

N = Total number of pair observations

The significance of correlation was tested by correlation table (Fisher and Yates, 1986).

Results and Discussion

Biometry of Udder

The overall least squares means of various udder parameters viz. udder length, udder width, udder depth and udder circumference of crossbred cows are presented in Table 2.

The overall mean udder length in crossbred cows was 65.23± 1.45cm. Effect of order of lactation on udder length was significant (P<0.01). Similar findings were reported by Mingoas et al. (2017) in Zebu cows. The udder length in cows of 6th lactation onwards (72.42 ± 2.01 cm) was significantly(P<0.05) higher than cows of 1st (61.61 ± 1.68 cm) and 2nd (63.38 ± 2.38 cm) lactation which did not differed significantly from each other. Increase in udder length with the advancement of lactation may be due to the physiological development of body and udder. Further decline in udder length may be due to shrinkage in udder with advancement of age. Influence of stage of lactation on udder length in crossbred cows was non-significant. Milk yield of cow increases during early stage of lactation and reaches peak at about 35-50 days and then declines gradually. With increase in milk yield, the udder length also increases. Hence, udder length may be higher during early stage of lactation.

The overall least squares mean of udder width in crossbred cows was 63.24 ± 1.45cm. Similar udder width was noticed by Patel et al. (2016) in crossbred cows (65.45 ± 0.70 cm). The difference due to parity in udder width was statistically significant (P<0.01). The udder width in cows of 6th lactation onwards was significantly (P<0.01) higher than cows of 1st to 5th lactation, which did not differed significantly from each other. The effect of stage of lactation on udder width in crossbred cows was non-significant.

Table 1 Least squares analysis of variance indicating effect of order of lactation and stage of lactation on udder measurement traits

Source of variation \ Traits	Udder length			Udder width			Udder depth			Udder circumference		
	d.f.	MSS	F	d.f.	MSS	F	d.f.	MSS	F	d.f.	MSS	F
OL	5	324.46	3.13*	5	991.49	5.09**	5	57.15	2.25	5	766.76	3.48**
SL	2	76.20	0.73	2	3.06	0.02	2	52.01	2.05	2	111.61	0.51
Error	102	103.64		102	194.68		102	25.37		102	220.37	

* P<0.05 ** P < 0.01

The mean udder depth recorded in crossbred cows was 19.03 ± 0.51cm. The effect of order and stage of lactation on udder depth in crossbred cows was non-significant.

The differences observed in udder circumference in different lactations were statistically significant (P<0.01). Perusal of data showed a gradual increase in circumference of the udder as the lactations increased up to 3rd which declined in 4th lactation and then again increased during 5th lactation onwards.

Associations among udder biometry traits

The udder length had positive and significant (P<0.01) correlations with udder width (0.78), udder circumference (0.58) and udder depth (0.58) in crossbred cows. The correlations of udder width with udder circumference (0.71) and udder depth (0.70) were positive and significant (P<0.01). Also the positive and significant (P<0.01) correlations of udder circumference with udder depth (0.52) was observed. These results indicated that all the udder measurement traits are highly associated with each other.

Udder morphological characteristics

A visual appraisal was made to record the shape of udder. Perusal of the data revealed that the frequencies of trough (bowl), round, goaty and pendulous type udder were 63.64%, 8.18%, 11.82% and 16.36 %, respectively. Thus, the trough type udder was observed in more than half of the cows. Present result was in agreement with Kamboj et al. (2007) who reported that trough shape udder was most frequent udder (45.94 %) in Karan Fries cows.

Various udder measurements in crossbred cows according to the udder shape

The mean udder length in crossbred cows ranged from 61.23 ± 1.20 cm in trough udders to 74.91 ± 1.86 cm in pendulous udder, udder width ranged from 56.65 ± 1.43 cm in trough udder to 77.40 ± 3.40 cm in pendulous udder. The udder depth ranged from 17.12 ± 0.54 cm in trough udder to 23.96 ± 1.24 cm in goaty udder. The udder circumference ranged from 80.93 ± 1.78 cm in trough udder to 103.06 ±1.75 cm in round udder. The differences observed in mean udder length, width, depth and circumference due to different shape of udder were statistically significant (P<0.01).

Overall mean udder length, width, depth and circumference in crossbred cows were 65.23 ± 1.45 cm, 63.24 ± 1.45 cm, 19.03 ± 0.51 cm and 86.49 ± 1.50 cm, respectively. It was noticed that all udder dimensions were highest in pendulous udder followed by round, trough (bowl) and goaty udder. The udder shape had significant (P<0.01) effect on udder length, udder width, udder depth and circumference. The udder length, udder width and udder depth of cows having pendulous, goaty and round udder was significantly (P<0.01) higher than udder length, udder width and udder depth of cows having trough shape udder. The udder circumference of cows having round shape udder was significantly (P<0.01) higher than udder circumference of cows having goaty and trough shape udder. The udder circumference of cows having round shape udder was at par with udder circumference of cows having pendulous udder.

Relationship between udder morphology and incidence of subclinical mastitis

Prevalence of subclinical mastitis in cows with different shape of udder showed higher occurrence of subclinical mastitis in pendulous udder (66.66%) followed by goaty (53.84%), round (44.44%) and trough (35.71%) shape udder (Table 3). The cows having pendulous udder had highest incidence of subclinical mastitis. This may be due to less distance between floor of udder

Table 2 Udder measurements of crossbred cows in different order of lactation and stages of lactation

Traits / Source of variation	N	Udder length (cm)	Udder width (cm)	Udder depth (cm)	Udder circumference (cm)
		Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.
Overall means (μ)	110	65.23±1.45	63.24±1.45	19.03±0.51	86.49±1.50
Order of lactation					
OL_1	42	61.61±1.68^{b}	56.92±2.10^{b}	17.18±0.75	80.60±2.28^{c}
OL_2	18	63.38±2.38^{b}	62.62±2.99^{b}	20.25±1.28	85.81±2.75^{abc}
OL_3	15	65.79±2.21^{ab}	64.39±2.97^{b}	19.13±1.29	93.52±3.63^{ab}
OL_4	7	66.43±4.77^{ab}	61.97±6.89^{b}	16.80±2.42	81.29±6.73^{bc}
OL_5	5	65.80±2.27^{ab}	61.20±3.83^{b}	19.18±1.64	83.30±10.91^{abc}
OL_6	23	72.42±2.01^{a}	75.81±3.27^{a}	22.04±1.06	95.46±2.93^{a}
Stage of lactation					
SL_1	5	72.3±3.43	66.64±8.12	18.38±1.05	85.96±9.20
SL_2	27	63.36±1.68	60.04±2.57	16.54±0.76	82.16±3.13
SL_3	78	65.42±1.29	64.27±1.77	19.93±0.64	88.02±1.72

Means under each class in the same column with different superscripts differ significantly (P<0.05 and P<0.01)from each other

and barn floor which increases the chance of entry of micro-organisms. Similarly, long and pendulous udders have more chances of injuries due to weak suspensory ligament which helps the pathogens to grow. Uzmay et al. (2003) reported that Holstein cows with pendulous udder had the highest risk ofsubclinical mastitis. Thus, selection of cows against pendulous shaped udder could help in reducing the incidence of subclinical mastitis in crossbred cows. Bharti et al. (2015) observed that the mean SCC level for pendulous udder was significantly (P<0.05) higher as compared to the regular shaped udder. Sharma et al. (2017) noticed that milk SCC was found to be significantly (P<0.01) higher in pendulous type udder and lower in bowl shaped udder in crossbred cows. Prevalence of subclinical mastitis in trough shaped udder was observed to be 35.71%. Hussain et al. (2012) reported 35.24% positive cases of SCM cows with trough shape udder which was in agreement with the present study.

Relationship between udder biometry and incidence of subclinical mastitis

The percentage of positive subclinical mastitis cases were found in cows with large udder length, udder width, udder depth and udder circumference category (61.11%, 52.17%, 52.94% and 47.22%). The large udder had more incidence of subclinical mastitis which may be due to the reason that large udder normally have wider area in contact with barn floor and had more chances of injury than the small udder. Further, when the cows are at rest, such udders gets spreads on the floor and gets contaminated which may be the possible cause for higher occurrence of subclinical mastitis.Udder depth had significant correlation with the mastitis. Higher the udder depth, lesser will be the distance of udder from floor making it vulnerable for risk of infection. In the present study, highest incidence (52.94%) of subclinical mastitis was found in cows with udder depth greater than 25 cm. Wattiaux (2005) reported that cows with too deep udder were more prone to mastitis or physical injuries.

Relationship between order of lactation and incidence of subclinical mastitis

The maximum occurrence (78.26%) of subclinical mastitis was in sixth and above parity cows. This may be due to the reason that older cows were milked more and thus exposed more to environmental pathogens that causes subclinical illness and more likely to have damaged teats or udder tissue in which contagious infection can easily enter and colonize. George et al. (2007) reported increased occurrence of subclinical mastitis with advancement of parity in crossbred cows.

Relationship between stages of lactation and incidence of subclinical mastitis

The highest occurrence of subclinical mastitis in crossbred cows was observed in late stage of lactation (46.15%) followed by early (40%) and mid (37%) stage of lactation. Higher prevalence of mastitis in late stage of lactation may be due to removal of protective keratin plug in teats. Tancin (2013) in dairy cows reported higher occurrences of subclinical mastitis in early and late lactation. Syridion et al. (2013) also reported higher incidence of SCM during late stage of lactation in Holstein Friesian crossbred cows.

Table 3. Incidence of subclinical mastitis in cows with different shape of udder

Udder shape	Frequency	Incidence of SCM	Percentage
Pendulous	18	12	66.66
Goaty	13	7	53.84
Round	9	4	44.44
Trough	70	25	35.71
Overall	110	48	43.64

Table 4. Udder biometry and incidence of subclinical mastitis

Parameter	No. of cows	Positive for SCM	Percentage
Udder length (cm)			
<55	18	5	27.77
55-75	74	32	43.24
>75	18	11	61.11
Udder width (cm)			
<55	33	11	33.33
55-75	54	25	46.3
>75	23	12	52.17
Udder depth (cm)			
<15	26	9	34.61
15-25	67	30	44.78
>25	17	9	52.94
Udder circumference	(cm)		
<75	28	12	42.86
75-95	46	19	41.3
>95	36	17	47.22

Table 5. Correlations of udder measurement traits with incidence of subclinical mastitis

Traits	Incidence of subclinical mastitis
Udder shape	0.24*
Udder length	0.19*
Udder width	0.16
Udder circumference	0.01
Udder floor to ground	-0.19*
Udder depth	0.27**
Lactation order	0.37**
Stage of lactation	0.01

*p<0.05 **p<0.01

Correlations of uddermeasurement traits with incidence of subclinical mastitis

The correlation coefficient of udder shape (0.24) with incidence of subclinical mastitis was positive and significant (P<0.05). Similar results were reported by Bharati et al. (2015) in crossbred cows. The results showed that with increase in udder length, there was increase in incidence of subclinical mastitis. The udder width (0.16) and udder circumference (0.01) had positive and non-significant association with incidence of subclinical mastitis.The correlation of udder depth (0.27) with incidence of subclinical mastitis was positive and significant (P<0.01). The results showed that lesser the udder depth, higher the cases of incidence of subclinical mastitis. This result was in line with the findings of Nemcova et al. (2007) in Holstein cows. The distance between udder floor to ground (-0.19) had negative and significant (P<0.05) correlation with incidence of subclinical mastitis. The results indicated that lesser the distance between udder floor to ground, higher the cases of subclinical mastitis.

Conclusions

The frequencies of trough (bowl), round, goaty and pendulous type udder in crossbred cows were 63.64%, 8.18%, 11.82% and 16.36%, respectively. Traits like udder shape, udder length and udder depth had positive and significant association with incidence of subclinical mastitis. Stages of lactation and higher lactation order were associated with increased incidence of subclinical mastitis.

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References

Bharti P, Bhakat C, Pankaj PK, Bhat SA, Prakash MA, Thul MR and Japeth KP (2015) Relationship of udder and teat conformation with intra-mammary infection in crossbred cows under hot-humid climate. Vet World 8: 93-98

Cerkascenko I (1958) Mol Mjasn Zivotn. Anim Breed. Abstr. 3:36

Fisher RA and Yates F (1986) Statistical table for biological, agricultural and medical research. Sixth Edn. Publishers Longman group Ltd England Pp, 63

George S, Joshi HC, Suman CL, Rathore RS and Bisht GS (2007) Incidence of subclinical mastitis in crossbred cattle herd. Indian J Anim Prod Manage 23: 1-4

Harvey WR (1990) Least squares analysis of data with unequal subclass number. USDA, ARS. 144

Hussain R, Khan A, Tariq JM. And Rizvi F (2012) Possible factors associated with mastitis in Indigenous cattle in Punjab, Pakistan. Pakistan Vet J 32: 605-608

Kamboj ML, Anshaj Singh and Shiv Prasad (2007) Effect of udder and teat shapes and their measurements on somatic cell counts in milk of Karan-Fries cows. Indian J Dairy Sci 60: 435-440

Kramer CV (1957) Extension of multiple range tests to group correlated adjusted means. Biometrics 13: 13-18

Mingoas KJP, Awah-Ndukum J, Dakyang H and Zoli PA (2017) Effects of body conformation and udder morphology on milk yield of zebu cows in North region of Cameroon Vet World 10: 901

Nemcova E, Stipkova M, Zavadilova L,Bouska J and Vacek M (2007) The relationship between somatic cell count, milk production and six linearly scored type traits in Holstein cows. Czech J Anim Sci 52: 437- 446

Patel YG, Trivedi MM, Rajpura RM, Savaliya FP, and Parmar M (2016) Udder and teat measurements and their relation with milk production in crossbred cows. Int J Sci Environ Technol 5: 3048-3054

Pyorala S (2003) Indicators of inflammation in the diagnosis of mastitis. Vet Res 34: 565-578

Rao GN (1985) Statistics for Agricultural Sciences Oxford and IBH Publishing Co New Delhi pp. 376

Sharma T, Das PK, Ghosh PR, Banerjee D and Mukherjee J (2017) Association between udder morphology and in vitro activity of milk leukocytes in high yielding crossbred cows. Vet World 10: 1-6

Syridion D, Layek SS, Mohanty TK, Kumaresan A, De, Kalyan, Manimaran A, Prasad Shiv and Venkatasubramanian V (2013) Effect of production systems on milk quality parameters in Holstein Friesian crossbred cows. Indian J Dairy Sci 66: 424-431

Tancin V (2013) Somatic cell counts in milk of dairy cows under practical condition. Slovak J Anim Sci 46: 31-34

Uzmay C, Kaya Y, Abbas Y and Kaya A (2003) Effect of udder and teat morphology, parity and lactation stage on sub-clinical mastitis on Holstein cows.Turk. J Vet Anim Sci 27: 695-710

Wattiaux MA (2005) Reproduction and genetic selection goals. Babcock Instt Int Dairy Res Dev 17: 1-6

Zdunczyk S, Zerbe H and Hoedemaker M (2003) Importance of oestrogen and oestrogen-active compounds for udder health in cattle: A review. Dtsch Tierarztl Wochenschr 110: 461

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RESEARCH ARTICLE

Economics of milk processing in cooperative sector of Haryana

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Abstract: In the rural sector, dairying and milk production is an important economic activity and has become a secondary source of income and employment. Cooperatives play an important role in animal husbandry and dairying sector, which contributes about one-fourth of agricultural GDP of the country. This study works out the cost of collection, chilling and transportation to arrive at economics of milk processing and manufacturing of milk products in Haryana by selecting milk plants from different zones in the cooperative sector. The economics of milk processing in Haryana show that procurement cost was found to be higher in Western zone which was estimated to Rs. 1.83 per litre of milk compared to Rs. 1.67 per litre in Eastern zone. Out of which transport cost accounted for maximum share (54.57%) followed by transportation cost (54.5 7%), chilling cost (24%), collection cost (17.1 4%) and reception cost (8.57 %). The highest profit was recorded in Dahi making which which yielded a profit over cost of 23.76 percent. The other higher profit giving products were double toned milk and ghee, which yielded profit over cost of 15.21 and 11.88 percent respectively. The profit margin for Paneer was found to be lowest at 7.76 % with cost of Rs 16.05 per kg. The information on the profitability of different products in Haryana can be handy to reorient the product mix and production scale by superimposing the cost of manufacturing and demand of different products over it.

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Introduction

The Dairy sector in India has grown substantially over the years. India ranks first among the world's milk producing nations, achieving an annual output of 186.40 million tonnes during the year 2018-19. About 80 million rural households are engaged in milk production with very high proportion of small & marginal and landless dairy farmers, (DAHD, 2020). In India, about 54% of the milk is available for sale to organized and unorganized players. Organized sector comprise of Government, Producers' Owned Institutions (Milk Cooperatives & Producer Companies) and Private players. Cooperatives play an important role in animal husbandry and dairying sector which contributes about one-fourth of agricultural GDP of the country (DAHD, 2020; Kumar, 2011). At present 210 Dairy Cooperative Milk Unions exist in the country which covers about 16.54 million farmers (20% of dairy farmers) under the ambit of 185,903 village level dairy corporative societies. According to a report by IMARC Group, the Indian dairy industry was worth a value of INR 9,168 Billion in 2018 and to reach a value of INR 21,971 billion by 2024 (IMARC, 2020). Dairy cooperatives are recognised as engine for the development of dairy industry in India because of their economic advantages, democratic character and social purpose. The cooperative sector, which balances the interests of producers and consumers, is best suited for dairy development. They possess inherent potentialities and in-built provisions to serve the cause of rural development.

Promotion of cooperatives is widely viewed as the most important institutional arrangement for spurring dairy development in India. Dairy cooperatives have led to a significant increase in both milk production and productivity, decrease in cost of milk production, reduce transaction costs of accessing inputs, information, technology, and markets and a realization of higher prices and profits (Kumar et al. 2011). Besides, dairy cooperatives have potential for joint development of innovations and sharing among members. In view of importance of dairy cooperative sector in

the industry this paper attempts to elucidate the performance of milk plants working under the ambit of dairy cooperative system in the state of Haryana.

Materials and Methods

Two milk plants having the highest milk handling performance from two NARP zones (Ghosh, S.P., 1986) of Haryana state, viz, Ballabhgarh plant from Eastern and Western zone were selected for the study. The milk plants were operating under the management of Haryana Dairy Development Cooperative Federation Ltd. with the brand name Vita. There are six milk unions operating under the federation in the state. The major products manufactured in both the plants include Pasteurized Liquid Milk, Ghee, Skimmed Milk Powder, Paneer, Dahi, Lassi and Kajupinni. The primary data during 2014 to 2015 pertaining to economic aspects from chilling units, Bulk Milk Coolers (BMC), Milk procurement societies and different sections of the milk plant were collected from the records of the plants, by taking the actual observations on temperature and quantity at the time of manufacturing of products and holding the interviews and discussion with the staff working in the plant. The collected data were analysed by using the methodology as follows:

Milk procurement cost=Cost of Collection + Cost of transportation + Cost of Chilling + Reception Cost

$$\text{Cost of collection per litre of milk} = \frac{\text{Total cost of milk collection}}{\text{Total quantity of milk collected}}$$

$$\text{Transportation cost per litre of milk} = \sum_{i=1}^{n} \frac{TCi}{MTi}$$

Where,

TC_i= Total transportation cost on the i^{th} route,

MT_i= Total quantity of milk transported on the i^{th}route

n = Number of transport routes

$$\text{Cost of chilling per litre of milk} = \frac{\text{Total cost of chilling}}{\text{Total quantity of milk chilled}}$$

The cost incurred on different components was considered for estimating the cost of products manufactured at the plants.

Steam

$$\text{Steam requirement (Kg.)} = \frac{M \times S \times T}{L}$$

Where,

M = Quantity of the product to be heated (kg.)

S = Specific heat of product

T = Temperature difference (T_1-T_2, initial and final temperature of the product in centigrade)

L = Latent heat of steam

Refrigeration load (kilo Cal) = M X S X T

Where,

M = Quantity of product to be cooled (kg)

S = Specific heat of the product

T= Temperature difference in centigrade (T_1-T_2) (Ahmed, 1997)

Results and Discussion

For calculation of cost of milk products manufacturing, there is a need to work out the cost of milk procurement and resource utilization in dairy plants.

Procurement cost

Milk procurement cost which includes cost incurred on collection, transportation and chilling of milk and reception, was estimated and presented in Table 1. The procurement cost was found to be higher in Western zone which was estimated to Rs. 1.83 per litre of milk compared to Rs. 1.77 per litre in Eastern zone. The transportation cost was dominant in both the zones which accounted for 43.72 and 66.47 percent of procurement cost in Western and Eastern zone respectively. The overall cost of milk procurement was found to be Rs. 1.81 per litre in Haryana. The

Table 1 Milk procurement cost of milk plants in Haryana (Rs./litre)

Sl. No.	Components	Eastern zone	Western zone	Over all
1	Collection cost	0.12(6.77)	0.48 (26.23)	0.3 (16.57)
2	Transportation cost	1.11(62.72)	0.80 (43.72)	0.96 (53.03)
3	Chilling cost	0.44(24.87)	0.40 (21.86)	0.42 (23.21)
4	Reception Cost	0.1 (5.65)	0.15(8.20)	0.13(7.19)
	Procurement cost	1.77 (100)	1.83 (100.00)	1.81 (100.00)

Figure in the parentheses are percent to total

studies done on similar aspects found procurement cost of milk in the similar range (https://www.vitaindia.org.in/milk-plants)

Resource utilization in dairy plants

Costs of different types of resource used in the plants were calculated and discussed below.

Cost of steam production

Steam is the root source of energy in the milk plant. The production of steam in the selected plants was10512 and 5840 metric tonnes in Eastern zone and Western zone plants respectively. Among the components of cost in producing steam, cost on coal was found to be highest in both plants with annual cost of Rs 2360.82 crores (59.08%). The other major costs involved in producing steam in both the selected plants were found to be labour and electricity which accounted for 8.89 and 7.93 percent respectively. The fixed cost in the study area was 5.05 percent to total cost or 0.105 Rs/kg of steam produced in the study area. The cost on depreciation of buildings and machinery was the considered as fixed cost while estimating cost of steam production. The other variable cost components involved in producing steam were water, softening of water and annual maintenance charges. The total variable cost for steam production accounted for 69.35 per cent of the total cost. Chauhan et al. (2015) corroborates the findings with a difference in the cost of steam production in Haryana. The difference found in the cost of steam production may be due to the cheap source of fuel being used in the studied plant. The plant used rice husk as a source of energy in most of the time and furnace oil in some cases as steam produced by rice husk is three times more that of furnace oil.

Cost of refrigeration

The overall total cost of refrigeration in the study area was Rs. 5808583 per year. The total variable cost contributes about 96 per cent whereas the rest was fixed cost. Among different component of cost, cost for electricity was highest (78 percent) followed by labour (10 per cent) which were the other major costs. Water and annual maintenance charges of refrigeration section shares 1.76 and 0.49 per cent respectively.

Cost of manufacturing of products

Product wise cost of manufacturing was worked out for the products like standardized milk, full cream milk, toned milk, double toned milk, paneer, ghee, Dahi and depicted in to variable cost which include cost of critical inputs/ resources such as Raw Material, Labour, Electricity, Water, Steam, and Refrigeration in addition to expenditure on Quality Control, Miscellaneous items, Packaging Material and Sundries and fixed cost comprising

Table 2 Component wise manufacturing cost of different products

Cost Components	Eastern zone		Western zone		Overall	
	Unit cost (Rs/L.)	Percentage cost	Unit cost (Rs/L.)	Percentage cost	Unit cost (Rs/L.)	Percentage cost
Standardized milk						
Total VC	36.04	99.78	34.95	95.81	35.52	97.77
Total FC	0.08	0.22	1.53	4.2	0.81	2.22
Total	36.12	100	36.48	100	36.30	100
Full Cream Milk						
Total VC	42.06	99.71	40.72	94.45	41.42	97.04
Total FC	0.12	0.29	2.4	5.57	1.27	2.96
Total cost	42.18	100	43.12	100	42.65	100
Double Toned Milk						
Total VC	27.82	99.72	27.26	92.76	27.56	96.14
Total FC	0.08	0.29	2.12	7.21	1.10	3.84
Total cost	27.9	100	29.39	100	28.65	100
Ghee						
Total VC	330.31	99.01	331.96	98.71	330.335	98.85
Total FC	3.31	0.99	4.34	1.3	3.83	1.14
Total cost	333.62	100	336.3	100	334.17	100.00
Paneer						
Total VC	234.77	99.77	200.84	97.94	217.795	98.91
Total FC	0.54	0.23	4.25	2.07	2.395	1.09
Total cost	235.31	100	205.09	100	220.2	100
Dahi*						
Total VC	16.03	100	10.35	100	13.19	100

*. In case of Dahi, fixed cost could not be ascertained due to non-availability of relevant data

Administration & Supervision and Interest & Depreciation (table-2).

Standardized milk

The component wise cost of manufacturing standardized milk was estimated and presented in Table 2. The manufacturing cost of standardized milk in the study area was found to Rs. 36.30 per litre. (Rs. 36.12 and Rs. 36.48 from Eastern zone and Western zone plants respectively). The variable cost alone accounted for more than 99 percent (99.78%) of total cost of manufacturing in Eastern zone where as it accounted to the tune of 95.81 percent in Western zone. The average proportion of variable cost in the study area was found to be 97.77 percent (table 2). The major component of fixed cost was found to be administration and supervision cost which was Rs. 0.08 (0.22%) and Rs. 1.37 (3.76%) per litre in the Eastern and Western zone respectively. The other components of fixed cost waswere interest and depreciation.

Full cream milk

:The variable cost accounted for Rs 42.06 (99.71 percent of total cost) and the fixed cost was merely 0.29 percent of total cost in Eastern zone. In the Western zone, it accounted for Rs 40.72 (94.45 percent of total cost) and the share of fixed cost observed was 5.57 percent. The other major costs observed were, Administration & Supervision cost, packaging material cost and labour cost which were estimated to be Rs. 1.97 (4.57%), Rs. 0.67 (1.55%) and Rs. 0.53 (1.23%) respectively. The overall manufacturing cost of full cream milk in the study area was estimated to be Rs. 42.65 per litre (97.04 percent) in the study area and the raw material cost was Rs. 39.58 (92.80%) as shown in Table- 2.

Double Toned Milk (DTM)

Total cost of manufacturing DTM was estimated to be Rs. 27.90 per litre in the Eastern zone out of which Rs.26.38 i.e., about 94.55 percent was raw material cost. The average variable cost was Rs. 27.56 per litre (96.14) and the fixed cost was Rs. 1.10 per litre (3.84%). The cost of manufacturing was higher in Western zone (Rs 29.39) where as in Eastern zone, it was only Rs 27.90. Overall, it was Rs 27.56 lit.

Ghee

Ghee was being manufacturing in the study area at Rs 330.34/ lit with hardly any difference over the zones (Rs 330.31 in Eastern zone and Rs 331.96 in Western zone). The variable cost constituted up to 98.80 percent of cost of manufacturing in the study area.

Paneer

On the other hand, in case of Paneer, cost of manufacturing was differing significantly between the zones. It was Rs 234.77/kg in Eastern zone and Rs 200.84/kg in Western zone. Overall, cost of manufacturing Paneer in the study area was found to be Rs 217.79/ kg.

Dahi

Total variable cost of Dahi was estimated at Rs 13.19 per pack of 200 ml in Eastern zone and Rs 10.35 in Western zone.The cost of manufacturing Dahi could not be bifurcated into variable cost and fixed cost because of unavailability of records on cost aspects with the plants.

Cost of critical inputs/ resources in the manufacturing of products

The study of usage of critical inputs and their costs in turn form the basis of strategic planning for the milk plants. So, the information was generated and presented product wise in table3.

Standardized milk

Among the different cost components, cost on raw material (raw milk) was dominant as it accounted for more than 90 percent of total cost on both the selected plants which is Rs. 34.52 (95.57%) and Rs. 33.16 (90.90 %) per litre in Eastern and Western zone plants respectively. The overall cost of raw material in manufacturing standardized milk in the study area was Rs. 33.84 per litre which was about 93.22 percent of total cost. The other major costs observed in manufacturing standardized milk was Refrigeration cost (1.39 %) as depicted in table – 3. The other minor cost components observed were Labour (0.23%), Electricity (0.12%), Steam (0.66%), Water (0.03%). The remaining expenditures can be ascertained to Quality Control, Miscellaneous items, Packaging Material and Sundries and fixed cost comprising Administration & Supervision and Interest & Depreciation.

Full Cream Milk

Component wise cost analysis revealed that raw materials alone accounted for 95.87 per cent of total expenditure in the selected plant of Eastern zone and 89.80 percent in Western zone. The overall contribution of critical resources can be presented as Raw material (92.8%), Labour (0.74%), Electricity (0.12%) Water (0.04%), Steam (0.55%) and Refrigeration (1.18%). These resources were used more in Western zone.

Double Toned Milk (DTM)

Overall, the main critical input ie raw materialcosted up to 89.98% in the manufacture of DTM. It costed more in Eastern zone (94.55%) than in Western zone (85.65%). The raw material was followed by refrigeration (1.78%), steam (0.84%) and labour

(0.37%). Other critical inputs were having very less and insignificant contribution (table-3).

Ghee

It is interesting to note that contribution of raw material ie milk found to be the 97.11% in Eastern zone and 95.15% in Western zone (overall, it was 96.36%). The zonal variation in this contribution might be because of difference and variation in the prices. In case of ghee, the other important inputs were found to be labour (0.56%) and electricity (0.16%) as shown in table-3.

Paneer

The picture of cost of key inputs in paneer manufacturing was no different from that of ghee. Here also, raw material ie milk contributed upto 95.46% of the cost of manufacturing followed by labour (1.39%) and electricity (0.16%) being other inputs contributing at very low level.

Dahi

Table 3 Cost of critical resources/ inputs in manufacture of different products (Rs per Lit/ kg)

Sl. No	Cost Components	Eastern zone		Western zone		Overall	
		Unit cost	Percent cost	Unit cost	Percent cost	Unit cost	Percent cost
Standardized milk							
1	Raw Material	34.52	95.57	33.16	90.9	33.84	93.22
2	Labour	0.09	0.25	0.08	0.22	0.09	0.23
3	Electricity	0.07	0.19	0.02	0.05	0.05	0.12
4	Water	0.01	0.03	0.01	0.03	0.01	0.03
5	Steam	0.14	0.39	0.34	0.93	0.24	0.66
6	Refrigeration	0.61	1.69	0.4	1.1	0.51	1.39
Full Cream Milk							
1	Raw Material	40.44	95.87	38.72	89.8	39.58	92.8
2	Labour	0.1	0.24	0.53	1.23	0.32	0.74
3	Electricity	0.08	0.19	0.02	0.05	0.05	0.12
4	Water	0.01	0.02	0.02	0.05	0.02	0.04
5	Steam	0.14	0.33	0.33	0.77	0.24	0.55
6	Refrigeration	0.61	1.45	0.4	0.93	0.51	1.18
Double Toned Milk							
1	Raw Material	26.38	94.55	25.17	85.64	25.77	89.98
2	Labour	0.07	0.25	0.14	0.48	0.11	0.37
3	Electricity	0.05	0.18	0.1	0.34	0.08	0.26
4	Water	0.01	0.04	0.02	0.07	0.02	0.05
5	Steam	0.14	0.5	0.34	1.16	0.24	0.84
6	Refrigeration	0.61	2.19	0.41	1.4	0.51	1.78
Ghee							
1	Raw Material	323.99	97.11	320	95.15	321.995	96.36
2	Labour	1.72	0.52	2	0.59	1.86	0.56
3	Electricity	0.41	0.12	0.64	0.19	0.53	0.16
4	Water	0.15	0.04	0.03	0.01	0.09	0.03
5	Steam	0.48	0.14	0.85	0.25	0.67	0.20
6	Refrigeration	0	0.00		0.00	0	0.00
Paneer							
1	Raw material	227.78	96.8	192.64	93.93	210.21	95.46
2	Labour	3.25	1.38	2.85	1.39	3.05	1.39
3	Electricity	0.12	0.05	0.58	0.28	0.35	0.16
4	Water	0.21	0.09	0.02	0.01	0.11	0.05
5	Steam	0.45	0.19	0.43	0.21	0.44	0.2
6	Refrigeration	0.45	0.19	0.22	0.11	0.33	0.15
Dahi							
	Raw material	12.03	75.04	5.97	57.69	9.00	67.91
	Processing charge	1.18	7.36	2.13	20.54	1.66	12.34

Table 4 Profitability in processing of milk in Haryana

Sl.No	Products	Eastern zone			Western zone			Overall	
		Price received by plant (Rs.)	Cost of manufacturing (Rs.)	Profit over cost (%)	Price received by plant (Rs.)	Cost of manufacturing (Rs.)	Profit over cost (%)	Cost of manufacturing (Rs.)	Profit over cost (%)
1	Paneer(kg)	237.5	235.31	0.93	235	205.09	14.58	220.20	7.76
2	Ghee(litre)	376	332.62	13.04	369	333.30	10.71	332.96	11.88
3	Dahi (200ml)	23.8	16.03	48.47	12	10.35	23.29	9.1825	23.76
4	Std milk (lit)	40.00	36.12	10.74	38	36.48	4.17	36.30	7.46
5	DTM (lit)	32.00	27.90	14.70	34	29.38	15.72	28.64	15.21
6	FCM(lit)	46.00	42.18	9.06	46	43.12	6.68	42.96	7.87
7	Chachh* (500ml)	8.8	7.61	15.64					
8	Toned Milk* (lit)	37.00	31.65	16.90					
9	Lassi*(200ml)				13	11.64	11.68		
10	Kajupinni*(900gm)				241	221.20	9.37		
11	SMP* (kg)				240	223.70	7.29		

*. Products produced in one plant only.

In case of dahi manufacturing, contribution of raw material was found to be 67.91% which was higher in Eastern zone (75.84%) than in Western zone (57.69%). The other processing charges (combining all other critical inputs) were 12.34% being higher in Western zone (20.54%) than in Eastern zone (7.36%). It seems that prices might be having a role in this contribution and causing variation between the zones.

Profitability of products

Profitability of the dairy products manufactured in Eastern zone is presented in Table 4. The information contained in the table reveals that Dahi turned out to be the most profitable product (48.47 per cent) followed by Toned Milk (16.90 per cent), Chach (15.64 per cent), Double Toned Milk (14.70 per cent), Ghee (13.04 per cent), Standardized milk (10.74 per cent) and Full Cream Milk (9.06 per cent). Among different products manufactured, Paneer manufacturing turned out to be the lowest profitable proposition (0.93 per cent). There is need to explore the possibility to increase the profits in Paneer manufacturing by increasing the price of the product or to adopt some suitable steps to use the residual whey for by products such as whey based beverages, whey powder and in pharmaceutical industry to bring down the cost.

Profitability of the dairy products manufactured in Sirsa milk plant(western zone) was worked out by comparing the unit cost with the unit price received by the plant for sale of different dairy products. The information contained in the Table 4 revealed that Dahi turned out to be the most profitable product (23%) followed by Lassi (19%), Toned Milk (24.76%), and KajuPinni (17.55%). The Paneer have the least profit over margin of 2 percent. The product wise profitability in processing milk in the study area was worked out and presented in Table 4. Comparatively higher profit over cost was found in processing milk to Dahi which yielded a profit over cost of 23.76 percent which was highest among the products. The other higher profit giving products were double toned milk and ghee which yielded profit over cost of 15.21 and 11.88 percent respectively. However higher profit margin was seen in case of ghee which was Rs. 39.54 per unit which was due to higher price of the product i.e., Rs. 372.5 Rs./ litre ghee. The profit margin for Paneer was found to be Rs. 16.05 per kg of Paneer. The profit margin for Dahi, Standardized Milk, Full Cream Milk and Double Toned Milk was found to be Rs. 3.14, Rs. 2.70, Rs. 4.36 and Rs. 3.35 per litre respectively.

Conclusions

The economics of milk processing in Haryana show that procurement cost was found to be higher in Western zone, estimated at Rs. 1.83 per litre of milk as compared to Rs. 1.67 per litre in Eastern zone. Out of the total procurement cost, transport cost formed of a major part (54.57%). Cost of manufacturing of different products was found to be lower in Eastern zone of the state except Paneer and Dahi where the case was reverse. The

major chunk of cost of manufacturing was taken by the variable cost and the fixed cost contributed up to 1.14% to 3.84% of the total cost. The contribution of critical inputs to the manufacturing cost show that major portion (upto 89.98% to 93.22%) goes to the raw materials in manufacturing of these products followed by refrigeration (0.00 to 1.39%), steam (0.2 to 0.84%, labour (0.23 to 1.39%) and water (0.03 to 0.05%). In case of Ghee and Paneer, Labour was found second most important component of cost of manufacturing after Raw materials.

The profitability of different products analysed show that comparatively higher profit over cost was found in processing milk to Dahi which yielded a profit over cost of 23.76 percent. The other higher profit giving products were Double Toned Milk and Ghee which yielded profit over cost of 15.21 and 11.88 percent respectively. The profit margin for Paneer was found to be lowest at 7.76 % with cost of Rs 16.05 per kg. So, Dahi and its different variants and Double Toned Milk can be produced to increase the profits from these plants. While coming to the operating cost scenario, proper technology and management in terms of the electricity and refrigeration cost is important to ensure higher profitability of the cooperative milk processing plants. The study showed that all the products manufactured in the milk plants are generating profits. The information on the profitability of different products in Haryana can be a useful tool for the Management to reshuffle the product mix of the plants on the basis of cost of manufacturing, profits and demand of different products.

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References

Ahmed, T (1997) Dairy Plant Engineering and Management, IVth Edition, Allahabad:Kitab Mahal, 22-A, Sarojini Naidu Marg, pp 48-51

Alli M, Chauhan, AK, Franco D, Singh S (2015) Economics of Resource Utilization for manufacturing of dairy products in a cooperative dairy plant in coastal Odisha. Indian J Econ Dev16:631-635

DAHD. 2020. Annual Report 2018-19. Dairy Development, pp 69-72

Kumar A, Staal SJ, Singh DK (2011) Smallholder dairy farmers' access to modern milk marketing chains in India. Agric Econ Res Rev 24: 243-253

IMARC (2020) The International Market Analysis Research and Consulting Group, retrieved from https://www.imarcgroup.com/indian-dairy-industry-cross-18599

Haryana Dairy Development Cooperative Federation Ltd (HDDCFL), retrieved from https://www.vitaindia.org.in/milk-plants

Mane SR (2013) Energy Management in a Dairy Industry. Proceedings of Institute of Research and Journals, International Conference, International Standard Book Number: 978-93-82702-25-2.

Modi A, Prajapat R (2014) Pasteurization process energy optimization for a milk dairy plant by energy audit approach. Int J Sci Technol. Res 3:181-188

Rangaswamy N, Dhaka JP (2007) Constraints faced by Co-operative and Private dairy plants in Tamil Nadu – A comparative analysis. Indian J Dairy Sci 60:300-306

TherajaBL,Theraja AK (1992) A Text Book of Electrical Technology, New Delhi: S. Chand and CompanyLtd.,pp-46

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RESEARCH ARTICLE

Emerging trend of Dairy based Producer Organizations: Case Studies from Himachal Pradesh

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Abstract: Dairy farming, in spite of being a major livelihood source in the hilly state of Himachal Pradesh, often faces many issues such as lower price realisation for milk. The organized milk marketing structure of the state is one of the weakest in the country. Lower remunerative returns have affected the attitude of farmers towards dairy farming. As a solution to such problems, innovative farmer organisations are making progress in the state. These organisations offer input services at lower prices at farmers' doorstep. They also buy milk at prices higher than prevailing rates. The reported evidences of dairy interventions through farmer producer organizations are still minimal from this region. The present study assessed the functioning of dairy based producer organisations in hills of Himachal Pradesh. Three farmer producer organizations engaged in community mobilization through Self Help Groups were selected for the study. The benefits accrued by members of these organisations range from input availability, extension advisory, credit support and veterinary services.

Keywords: Dairy farming, Himachal Pradesh, Producer organisations

Introduction

Dairy farmers in the hills of Himachal Pradesh toil hard to secure livelihood from livestock keeping. Out of total 14, 83,280 households in the state, 52.65% own dairy cattle (GOI, 2019; HP, 2018). The gradual decrease in landholding size has necessitated livestock keeping among households. The share of small and marginal farmers to total milk production in the state has increased from 89.9 % in 2003 to 92.7% in 2013 (Kumar et al. 2018). These smallholder farmers face numerous challenges in dairy production. Dairy sector remains underdeveloped and performs much below its full potential. The average dairy animal productivity is lower than the national average and even lower than adjoining hill state of Uttarakhand (Table 1). In addition to this, the milk price fetched by farmers across various marketing channels is one of the lowest in the country (Kumar et al. 2018). Poor bargaining power and difficulties in transportation forces farmers to sell milk at lower prices (Pathania and Sharma, 2016). The state has one of the weakest organised milk marketing structure (Kale et al. 2016) and the milk procurement by co-operatives in the state is very low (1.76%) (Table1). The lower returns have affected the attitude of farmers towards dairy farming and the farmers are reluctant to sell milk leading to lowest marketed surplus. So, the activity remains non commercialized in the state (Dogra, 2016). The dairy processing activities by state owned milk marketing organisations have considerably underperformed(Kumar,2018) and has limited marketing channels across or outside state(TOI,2018).Low performance of state owned dairy co-operatives has given way towards innovative Farmer Producer Organizations (FPOs). These organisations combine the business principles of company and welfare aspects of co-operatives. There are evidence of better price realisation when farmers join together to form dairy based producer organisations (Jose et al. 2019a).Producer organization is a generic name that represents various cooperatives, self help groups, federation of SHGs, commodity interest groups, farmers club, producer company etc.(NABARD,2005; Jose et al. 2019b).The reported evidences of dairy interventions through producer organizations are still very few in India in general and specifically in the state of Himachal Pradesh. With this background, this paper examines the functioning of three producer organizations of the state.

Materials and Methods

The present study was purposively conducted in the state of Himachal Pradesh. Three districts (Kangra, Bilaspur and Solan) of lower regions of the state were selected. Farmers of these regions have been traditionally engaged in rearing cattle. In each

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district, one prominent farmer organisation was purposively selected. The primary data were collected from board of directors and members of these organisations. Semi-structured interview schedule and focused group discussions methods were used to collect data. The data was also supplemented through telephonic interviews with the managers, project officers, cluster co-coordinators and board of directors of these organisations. Secondary data were collected through reports of these organizations.

Results and Discussion

Kamdhenu Farmer & Consumer Welfare Forum, Bilaspur

This dairy farmers' organization works in district Bilaspur, Himachal Pradesh. In 2001, the organisation started its work as association with 8-10 farmers with collection of 35-40 liters of milk in a day. At the time of data collection, organisation collects milk from 4700 families in 350 villages of 65 village panchayats. It runs five milk chilling centers and a central milk processing plant at Namhol, Bilaspur. Daily 31000 liters of milk is procured from 4700 families. This is substantial quantity, considering the fact that state milk marketing federation procures 70,000 liters of milk per day (HP, 2018). The milk procurement price offered by the organization (Rs 33.63 (4.1 % fat & 8% SNF)) is higher than the price provided by state milk marketing federation (Rs 27.80 per litre).It also ensures doorstep availability of cattle feed and feed supplements. The organisation has received support from District Rural Development Agency (DRDA), NABARD and state government to set up milk processing infrastructure.

Incentives for higher milk production

Milk producers are incentivized through bonus payments for increasing milk production (additional payment of Rs.25 per liter of milk for more production beyond 10 liters of milk).Milk producers who bring more than 10 liters of milk to the society meet on monthly basis with the representatives of society to discuss issues and problems. Dairy farmers avail doorstep delivery of veterinary services during normal, odd hours and holidays from society. They also save their income from milk selling and avail loans at times of need from saving and credit unit of the society .The society uses Self Help Group approach for community mobilization. A cluster co-coordinator overlooks the formation of 15-20 SHG of farmers and manages them.

Amrit Dhara Farmer Producer Organisation, Darlaghat, Solan

This organisation has been supported by Ambuja Cement Foundation under Corporate Social Responsibility (CSR) program (Mahajani, 2016). Initially, the foundation trained a group of women dairy farmers with the support of veterinarians of state animal husbandry department. The training process initially focused on breeding and feeding management (Sharma, 2018). Later on, training was given in primary animal health care practices also so that the women can act as Pashu Sakhis in the community. In 2016, these women farmers formed a dairy FPO under co-operatives societies' act. The FPO now operates with 206 members in 60-70 villages.

Feed and implements support

The society acquired dealership of cattle concentrate feed. Bulk purchases by society helps it to reduce transportation and storage costs. Animal health volunteers ensure feed demand estimates at their respective village. This helps in direct wholesale purchase and sometimes direct transportation to farmers. Therefore, storage costs have been reduced and farmers receive feed at their doorstep. The society operates at lower price margin than local feed sellers. Farmers save Rs. 100 per quintal of feed and receive it at their doorstep. The region faces acute fodder scarcity as landholdings are limited. To solve this issue, the society

Table 1 Dairy production scenario in hills of Himachal Pradesh

Production Parameters	Himachal	Uttrakhand	All India
i)Milk Production(Thousand Tonnes)	1460.15	1792.37	187749.46
ii)Per capita availability	565 gm/day	455 gm/day	394 gm/day
iii)Total number of villages	17,882	15,745	649481
iv)Total number of milk potential villages	5290(29.58%)	4610(29.28%)	3,20,000(52.25%)
v)Milch animal owning households	7,81,000	9,01,000	69100000
Livestock Productivity (Kg/Day)			
i) Cattle			
Exotic	9.24	11.01	11.67
Crossbred Exotic	5.00	7.13	7.85
Indigenous	3.58	4.76	3.85
Non descript	1.96	1.85	2.50
ii)Buffalo			
Indigenous	4.43	5.33	6.34
Non-descript	3.26	4.01	4.35

Source:GOI,2019

buys fodder in bulk from adjoining states and sells at lower price (Rs. 7-8/kg) than the fodder bought individually (Rs. 10-11/kg) by the farmers. The society keeps its margin for operational expenses. Members can buy farm implements also (chaff cutters, farrows) from the shop owned by the society.

Collective Milk Marketing

Amrit Dhara, dairy FPO collects 700 liters of milk every day and sells milk to neighboring town. The milk procurement price is Rs 31 and sold at Rs 38 per litre. Though the price received may appear lower, farmers can sell milk at their doorsteps. Mostly milk is sold as loose milk and provides *Dahi* and *Paneer* on demand. The organisation has opened a bulk milk cooling unit to store milk and now is planning to open to milk processing unit.

Self Help groups of Tata Himmotthan Society, Kangra

The Himmotthan society, an associate organisation of TATA trust, works in Shahpur and Baijnath blocks of district Kangra, Himachal Pradesh. The organization started its work in 2018 and has created 43 SHGs with 485 members. The basic unit of organizational work is individual families. One woman dairy farmer from each family is selected while forming SHG with 8-12 members.15-20 such SHG form part of village cluster. Activities of 10-15 SHG's of each village cluster are managed by village co-coordinators. These functionaries ensure activities needed for smooth functioning of SHG by conducting regular meetings, record keeping and encouraging regular saving and lending.

Community Fodder Interventions

The organisation has been working for development of fodder base in wastelands and community lands. The SHG members are encouraged to use clean wastelands through MGNREGA and improved fodder varieties are grown and harvested them. Recently, the organisation is working with veterinary college Palampur and NABARD and they support these interventions on a larger scale. Besides this, support for building improved cattle houses on cost sharing basis (50:50) have been initiated among the community.

Comparative evaluation of services received through Dairy Producer Organizations

Input Services

As evident from table 3, farmers of Vyas Kamdhenu Farmer and Consumer Welfare Forum received input support in the form of feed and fodder seeds at lower prices at doorsteps. Farmers of this organisation received cattle feed, while farmers of Himotthan society received fodder seeds. Vyas Kamdhenu Farmer and Consumer Welfare Forum, being a bigger organisation offers more benefits to producer members.

Veterinary service provision

Table 2 Comparative evaluation of services received by Dairy Producer Organizations

	Vyas Kamdhenu Farmer & Consumer Welfare Forum, Bilaspur	Amrit Dhara FPO, Darlaghat, Solan	Himotthan Society, Kangra
I)Input Provision			
Input supply at lower Rates	Yes	Yes	Partially yes
Input supply at door step	Yes	Yes	No
High quality of inputs	Yes	Yes	Yes
Provision of extension information	Yes	Yes	Yes
Provision of veterinary services	Yes	Partial	No
II)Financial Services			
Financial education	Yes	Yes	Yes
Savings	Yes	Yes	Yes
Credit	Yes	No	No
III)Milk Marketing			
Products	Milk, Paneer, Ghee, Curd	Milk	No
Market channels	Sold to major towns and districts of State and Chandigarh	Local Shopkeepers	No
Milk Storage	Yes	Yes	No
Milk Transport	Yes	Yes	No
Milk processing(Sold in packets)	Yes	No	No
Value addition(Ghee/Paneer)	Yes	No	No
Branding	Yes	No	No

Considering veterinary service provision, Vyas Kamdhenu Farmer and Consumer Welfare

Forum offers timely veterinary services to producers. Amrit Dhara FPO provides basic veterinary aid to members through trained women primary animal health workers and depend on state veterinary institutions for major services. Synergisms in activities of both have been seen in vaccination where women members of FPO guide dairy farmers for vaccination and even aid in cattle handling. Himmotthan society does not provide such services from their organisation and depend on government veterinary institutions. However, regular animal health camps are organized by society through state animal husbandry department and veterinary college Palampur.

Extension and advisory services

The extension advisory services are offered by conducting regular discussions on various animal husbandry topics in monthly group meetings of Vyas Kamdhenu Forum and Amrit Dhara FPO. Himmotthan society organises regular extension advisory camps in collaboration with state veterinary college.

Financial services

Members of Vyas Kamdhenu Farmer and Consumer Welfare Forum receive benefits of financial education, savings from their sale of milk as well as credit for their dairy and other requirements. This organisation has a credit unit to meet these requirements of farmers. Amrit Dhara and Himmotthan society offers financial education and ensures regular saving and self lending among members.

Milk marketing

Vyas Kamdhenu Forum sells milk and other processed products like paneer, ghee and curd to major towns and districts of Himachal Pradesh. Milk processing and value addition is being done by the forum. It runs five milk chilling centers and a central milk processing plant at Namhol, Bilaspur. Amrit Dhara FPO sells milk and prepares Dahi and Paneer only on demand basis. It has a chilling centre of 2000 liters capacity. Members of Himmotthan society do not receive any support on milk marketing till now.

Conclusions

The three FPOs considered in the present study are successful in addressing different challenges of dairy farmers in the state. However, their performance and scale of operation depend upon number of years in operation and membership size. Among all these farmer organisations, Vyas Kamdhenu Forum was established earlier and has a larger farmer member base. Therefore, it works on a larger scale with well better input support and established milk marketing to farmers. Amrit Dhara FPO has gradually increased its foray into milk marketing and processing. Himmotthan society has focused on input interventions to improve dairy development in the region. Thus, it can be concluded that during initial stages of formation of FPO, the benefits accrued may be limited to input availability. Later on, with larger member base, the scale of operation widens up and newer activities of dairy value addition, provision of veterinary services, dairy extension and credit facilities to the farmer members are offered. Investing sufficient time and effort in mobilizing the farmers through SHGs, sound leadership, transparency in operations, accounting, record keeping, and resource management are key factors determining the efficiency and effectiveness of these organisations. It is imperative that such types of Farmer Producer Organisations are encouraged to improve the state of dairy development in hills of India.

References

Dogra AK (2016) Dairy Development in Himachal Pradesh. J Adv Sch Res All Edu 12: 366 - 368

GOI (2019) State Dairy Profiles, Bimonthly report, 2019, Dairy Development Schemes, Department of animal husbandry and dairying, Ministry of Fisheries, animal husbandry & dairying, Government of India, New Delhi. pp 66

HP (2018) Statistical abstract of Himachal Pradesh, Department of Economic and Statistics, Government of Himachal Pradesh, Shimla. pp 49

Jose E, Meena HR, Meena BS (2019a) Genesis of dairy based farmer producer companies in Kerala. Indian J Dairy Sci 72: 218-222

Jose E, Meena HR, Verma AP (2019b) Case Studies of Dairy Based Farmer Producer Companies in Kerala. Int J Curr Microbiol App Sci 8: 501-505

Kale RB, Ponnusamy K, Chakravarty AK, Sendhil R, Mohammad A (2016) Assessing resource and infrastructure disparities to strengthen Indian dairy sector. Indian J Anim Sci 86: 720–725

Kumar A, Mishra AK, Parappurathu S, Jha, GK (2018) Farmers' Choice of Milk-marketing Channels in India. Econ Polit Wkly 53: 59

Kumar S (2018) Himachal: Dairy overhaul, The Statesman, Shimla, November 12, 2018 Available as https://www.thestatesman.com/india/himachal-dairy-overhaul-1502707198.html

Mahajani A (2016) Stakeholder Engagement for a Sustainable Initiative: A case of a project by ACF focusing on women veterinary care providers in Darlaghat, Himachal Pradesh, India. OIDA-Int J Sustainable Dev 9: 103-114

NABARD (2005) Farmers' Producer Organisations. Frequently Asked Questions (FAQs). National Bank for Agriculture and Rural Development, Mumbai pp. 4–5

Pathania, MS and Sharma A (2016) Economic analysis of milch animals in Jaisinghpur tehsil of district Kangra. Him J Agric Res 42: 37-46

Sharma S (2018) Women Empowerment through Cooperatives. A Case Study of amrit Dhara Milk Producers Marketing Co-operative Society Ltd. Darlaghat, Himachal Pradesh A newsletter of Centre for Professional Excellence in Cooperatives (C-PEC), BIRD, Lucknow Volume III. pp 3-5

Singh R, and Vaidya CS (2002) Smallholder dairy farming in Himachal Pradesh, India characteristics, constraints and development opportunities (Eds) Tulachan PM, Jabbar, MA, and Mohamed Saleem MA. Smallholder Dairy in Mixed Farming Systems of the Hindu Kush-Himalayas, ICIMOD Kathmandu. pp 99

TOI (2018) NDDB to promote dairy farming in Himachal Pradesh, Vadodara, The Times of India, June 9,2018 Available as

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RESEARCH ARTICLE

Impact of feed optimization and extension intervention on productive and reproductive performance in commercial dairy farms of urban areas in the eastern region of Ethiopia

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Abstract: This study aimed at assessing the husbandry practices and evaluating the impact of feed optimization on productive performances and reproductive disorders of dairy cows. The study was conducted on purposefully selected 76 and 16 dairy farms for pre-and post-intervention study, respectively, from Harar and Dire Dawa cities. Data were collected by semi-structured questionnaires and focused group discussion starting from October 2018 to September 2020. Commonly used feeds were standardized to the cows' requirement. Milk yield was recorded every day for four consecutive months while milk composition was evaluated every month. Data analysis was done by SPSS, one-way ANOVA, and t-Test. The pre-intervention results showed that farmers from the small, medium, and large-scale farms fed cows on low concentrate quantity of 3.76, 4.61, and 5.84Kg/day, respectively. The quantity of concentrate supplementation per cow per day was significantly different ($p<0.0001$) among farm-scales. Cows maintained under different farm-scales produced low milk yields (9.57-13.07 litter). Cows from large-scale farms significantly produced milk with lower fat ($P<0.0006$) whereas small-scale farms produced milk with lower protein and milk urea ($P<0.0001$). All milking cows maintained in small, medium, and large farm-scales were highly affected by anestrus (44.45%), placenta retention (35.19%), and repeat breeding (20.37%). The technical intervention of feed optimization boosted daily milk yield from 17.64 to 27.44%, enhanced most milk components except fat and milk urea, and reduced the incidence of anestrus (11.11%), retention of the placenta (5.56-11.11%), repeat breeding (11.11-22.22), dystocia (5.56-11.11%) and prolapse (5.56%). Finally, this study concluded that regular technical advice and feed optimization improved milk production and reduced reproductive disorders of dairy farmers in all farm categories.

Keywords: Cows, Farm-scales, Intervention, Milk production, Reproductive disorders

Introduction

In Ethiopia, market-oriented dairy farming primarily contributes to the supply of milk and milk products in urban centers (Mekonnen et al. 2006). The Ethiopian government has recently given considerable attention to the need to increase milk production (Belay and Geert, 2016). For this purpose, the government consistently introduced improved breeds and semen to dairy farmers. These genetically improved breeds are mostly kept in the urban area of the country due to the adequate availability of feeds and veterinary services (Azage et al.2013). The urban commercial dairy producers would rely almost exclusively on artificial insemination for better semen and pay for the more expensive imported genetics and breeding supplies. Despite this, cows reared under different commercial farm-scales were producing less daily milk, 5–15 litres (Azage et al. 2000), and total milk production per lactation, 3208.56 litres (Dessalegn et al. 2016) which is by far lower than 5807 litres reported in other African countries (Naceur et al. 2012). The lower milk production under commercial farms can be attributed to many constraints, but the most important factors repeatedly raised are the animal's genetic potential and feed (Belay and Geert, 2016). However, so many dairy farmers claim that poor breed quality has much more adverse effect on milk production than inadequate feeding. Conversely, others (Van Marle-Köster and Webb, 2014) suggested that the improvement of the genetic potential of dairy cattle increases milk yield if feed management skills are acquired and animal health care services are accessed by dairy farmers. For this reason, this research was done to examine the effect of feed optimization along with training, advice, and proper follow-up action on milk production and composition and reproductive disorders of dairy farms with different husbandry practices and

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farm-scales. Therefore, the objectives of this study were to assess the husbandry practices and standardize feed to the requirements of peak stage lactating cows and evaluate its impact on milk yield, milk composition, and reproductive disorders of lactating cows maintained under different husbandry and farm sizes.

Materials and Methods

Baseline and post-intervention study

The study was conducted in the eastern region of Ethiopia in and around Dire Dawa and Harar cities. Based on their willingness to engage in training and share experience at Haramaya University dairy farm, 23 small-scales (owned milking cows ≤ 10; 10 from Dire Dawa and 13 from Harar), 34 medium-scales (owned milking cows between 11 to 20; 18 from Dire Dawa and 16 from Harar), and 19 large-scales (owned milking cows >20; 12 from Dire Dawa and 7 from Harar) were purposefully selected. Survey data on husbandry practices were collected using semi-structured questionnaires, detailed assessment of farm notebook records, and focused group discussion (FGD). A total of 152 Holstein Friesian with Zebu crossbred cows (HFCC) within 3-5 parity, two from each farm were deliberately selected based on their population and assessed for their reproductive disorders, milk yield, and composition. A format was distributed to each farm to record data on milk and reproductive disorders by the farm head and was collected monthly. Reproductive disorders such as retained placenta, dystocia, anoestrus, repeat breeder, and prolapse that occurred to each cow in each farm were recorded based on existing definition (Adane et al. 2017). All identified dairy farm owners and/or their representatives were invited and trained on dairy production and management and shared practical skills at the Haramaya University dairy farm. Then, each farm was visited once monthly for four consecutive months during the pre-intervention to crosscheck the actual and adapted practices regardless of their farm-scales. However, only 16 farm owners (8 medium-scale; 4 from Dire Dawa and 4 from Harar, and 8 large-scales; 4 from Dire Dawa and 4 from Harar) who fulfilled all the required quantity and necessary feed ingredients used in ration formulation were purposefully considered in the post-intervention assessment. The post-intervention assessment was done on 48 HFCC (3 milking cows from each farm) which were purposefully selected based on their similar pregnant stage and parity then monitored starting from three weeks before calving to 120 days post-calving. The advice was given to all farm attendants to feed their cows separately twice a day at a rate of 0.5 kg per 1 kg of milk (Pandey and Voskuil, 2011), provide water and roughage feeding *ad libitum*, and to measure daily feed and milk produced.

Nutritional analysis and ration formulation

Milk sample was taken every month from each cow (100 ml) and pooled for cows in the same farm and separately analysed in duplicate for composition in the morning and afternoon by Milko-Scan™ FT1. Commonly provided feed was collected from each farm and samples of the same feed type were pooled, thoroughly mixed, sub-sampled (500 g) and analysed (AOAC, 2000) for their nutritional content. Accordingly, a ration was formulated from ground maize, wheat bran, wheat short, soybean meal, peanut meal, salt, ruminant premix, and di-calcium phosphate by feedWin at a proportion of 29, 31.2, 18, 5, 15, 0.8, 0.5, and 0.5%, respectively, so that it contains 18.02 crude protein and 61.2% TDN. Thereafter, the format that indicated this feed proportion was printed and distributed to all farms considered in the post-intervention so that they could make their ration at their farm with the same ingredients and amounts.

Statistical analysis and model

All the survey data were analysed for percentage and means by SPSS version 20. The concentrate supplementation, milk yield, and composition data were subjected to one-way ANOVA of SAS version 9.4 while the comparison data were analysed by t-Test Paired for two samples for means to recognize the impact of the technical-intervention. The statistical model: $Yij = \mu + Fi + eij$; where: Y_{ij} is the J^{th} observation of the i^{th} farm-scale; μ is the population mean; F_j is farm-scale (small, medium, and large); and e_{ij} is the random residual effect. The existence of significant effect among farms was separated by Duncan's multiple range tests at 5 % level of significance.

Results and Discussion

Pre-intervention husbandry practices

Across all the farm categories in the study area, Friesian crosses accounted for 82.8 percent and were the largest proportion of the herd maintained by farm owners due to higher milk productivity, but the majority of the farm owners did not know their exact crossbreed levels. Small size dairy farmers keep relatively many local cow breeds in their herd (18.7%) due to the lower feed requirements and a higher concentration of milk solids. Besides, small-scale dairy farming is their ancillary occupation (88.7%) unlike the medium-scale (91.6%) and large-scale (100%) which are running their farm as the main business.

Natural mating was the most (67.5%) practised breeding method in all the study farms whereas AI services were utilized mostly (31.4%) by small-scale farms with a desire to improve the genetic potential of their animals by superior bull's semen. The majority (95.60) of dairy farmers did not keep proper records relating to the date of birth, breeding, vaccinations, past health problems, treatment given, and daily milk yield for individual cows. Almost all farms (95.2%) were managed by owners who did not possess the relevant professional training and this was the cause for a large number of farms being managed in traditional ways with little or no improved management practices. This is in line with

the result indicated by Ponnusamy et al. (2019) and Patel et al. (2020).

Concentrate feeding practices and levels

A majority of dairy farmers fed their milking cows on concentrate at different proportions across all the farm categories (Table 1). The quantity of concentrate supplementation per cow per day was significantly different ($p<0.0001$) among farm-scales, whereas the quantity of concentrate supplementation per liter of milk was not. The amounts of concentrate used in large-scale farms were higher than in other farm sizes. This indicates that they give more care to their cows as they have comparative advantages in milk production which is consistent with the study reported by Gelila (2017). However, quantities used in all farm sizes were lower than the recommended, 6.5-10.5 kg/day (Kavanagh, 2015). The compound concentrate feeding based on milk yield was also below the recommended, 0.5 kg/kg of milk, which is the point of biological and economic optima (Pandey and Voskuil, 2011).

Milk yield and composition

Mean daily milk yield at peak lactation stage under different farm-scales was significantly different ($p<0.0001$) (Table 1). The overall recorded daily milk yield per cow in all the farms in the study was lower than the expected level (20 liters) reported in an earlier study for crossbred cows in Ethiopia (Azage et al. 2000). Cows from the small-scale farms were produced lower yields than cows from the medium and large farm-scales and this is consistent with earlier study reports (Gelila, 2017). This could be due to lower concentrate level utilization. Cows from the large-scale farms were significantly produced milk with lower in milk fat ($P<0.0006$) than cows from the other farm-scales, whereas cows from the small-scale farms were produced milk with lower protein and milk urea (MU) ($P<0.0001$) than cows from the other farm scales (Table 1). The fat and solid not fat components from cows of all farms were higher than the lower limit 3.5% and 8.5%, respectively.

Major reproductive problems

Anestrus, retention of placenta, and dystocia were prevalent among the reproductive disorders (Table 2) and this corresponded to results reported earlier (Khan et al. 2016; Patel et al, 2020). Cows maintained under small-scale farms were more affected by anestrus, retention of the placenta, and repeat breeding. This might be due to the lower quantity of concentrate supplementation (Table 1) and the intensive use of AI. The overall prevalence of repeat breeding and anestrus in this study was within the range of 5-30% (Patel et al, 2020; Gupta and Deopurkar, 2005; Hunduma, 2013) and 10-40% reported by Zdunczyk et al. (2002), respectively. The incidence of placenta retention in this study was higher than 18.3% which was reported by Degefa et

Table 1 Pre-intervention concentrate supplementation and milk yield and composition (n=76)

Parameters	Farm scales			P-value	SL
	Small (n=23)	Medium (n= 34)	Large (n= 19)		
Amount of concentrate offered (Kg/cow/day)	3.76^{c}	4.61^{b}	5.84^{a}	<0.0001	***
Concentrate supplement per liter of milk (Kg/litter)	0.39	0.41	0.45	0.4566	NS
Milk yield (Liter/cow/day)	9.57^{c}	11.29^{b}	13.07^{a}	<0.0001	***
Milk composition					
Fat (%)	4.40^{a}	4.00^{ab}	3.60^{b}	<0.0006	***
Protein (%)	3.00^{b}	3.81^{a}	3.97^{a}	<0.0001	***
Lactose (%)	4.72	4.70	4.66	0.507	NS
Solid not fat (%)	8.50	8.56	8.54	0.960	NS
Total solid (%)	12.86	12.00	11.91	0.067	NS
Milk Urea (mg/dl)	15.30^{b}	30.01^{a}	30.10^{a}	<0.0001	***

a ,b ,c- Different superscripts in a row are significantly different at P<0.05.NS=Non-significant; SL=Significant level

Table 2 Major reproductive disorders assessed during pre-intervention study (n=76)

Reproductive disorders	Farm Scales (%)			Overall mean
	Small (n= 23)	Medium (n= 34)	Large (n= 19)	(n= 76)
Anestrus	88.89	27.78	16.67	44.45
Retention of placenta	77.78	16.67	11.11	35.19
Repeat breeding	27.78	11.11	22.22	20.37
Dystocia	0.0	5.56	11.11	5.56
Prolapse	0.0	0.0	5.56	1.85

n= number of sample farms

Table 3 Least mean squares comparison of milk yield and composition for pre- and post-intervention study

Parameters	Farm Scale	Pre-In(n=76)	Post-In(n=16)	P-value	SL	Δ Change (%)
Milk yield (L)	Medium	11.29[b]	15.56[a]	<0.0001	***	4.27 (27.44%)
	Large	13.07[b]	15.87[a]	0.0007	***	2.80 (17.64%)
Milk composition (%)						
Fat	Medium	4.00[a]	3.64[b]	0.004	**	- 0.36 (9.89%)
	Large	3.60[a]	3.58[a]	0.920	NS	- 0.02 (0.56%)
Protein	Medium	3.81[a]	3.93[a]	0.060	NS	0.12 (3.05%)
	Large	3.97[a]	3.98[a]	0.920	NS	0.01 (0.25%)
Lactose	Medium	4.70[a]	4.73[a]	0.447	NS	0.03 (0.63%)
	Large	4.66[a]	4.69[a]	0.502	NS	0.03(0.64%)
Solid not fat (%)	Medium	8.56[b]	9.30[a]	0.024	*	0.73 (7.85%)
	Large	8.54[b]	10.10[a]	<0.0001	***	1.56 (15.45%)
Total solid (%)	Medium	12.00[b]	13.40[a]	0.007	*	1.4 (10.45%)
	Large	11.91[b]	12.90[a]	0.002	*	0.9 (6.98%)
Milk Urea	Medium	30. 01[a]	20.70[b]	<0.0001	***	- 9.31 (44.96%)
(mg/dl)	Large	30.10[a]	22.20[b]	<0.0001	***	- 7.90 (35.59%)

n=number of sample farms, Pre-In=Pre-intervention, Post-In=Post-intervention, SL=Significance level, NS=Non-significant (p>0.05), Δ=Performance change

Table 4 Influence of technical-intervention on some reproductive disorders

Variables	Farm Scale	Pre-In(n=76)	Post-In(n=16)	Δ changes (%)
Anestrus	Medium	27.78	16.67	11.11
	Large	16.67	5.56	11.11
Retention of	Medium	16.67	11.11	5.56
placenta	Large	11.11	0.00	11.11
Repeat breeding	Medium	11.11	0.00	- 11.11
	Large	22.22	0.00	- 22.22
Dystocia	Medium	5.56	0.00	- 5.56
	Large	11.11	0.00	- 11.11
Prolapse	Medium	0.00	0.00	0.00
	Large	5.56	0.00	- 5.56

n= number of sample farms, Δ= Performance change, Pre-In=pre intervention, post-In=post-intervention

al. (2011). The higher incidence of dystocia noted in the large-sized farms might be due to the heavy grain feeding practices which are in line with the study reported by Grimard et al. (2006). These variations in the prevalence of reproductive health problems might be due to the differences in the husbandry system and genetic blood level of cows in each farm.

Post-intervention husbandry practices

The intervention practice through feed optimization significantly (P<0.0001) increased daily milk yield as compared to pre-intervention assessment (Table 3). This response indicates the higher potential of cows if they feed on the required quantity of balanced ration. In agreement with this study, an increased milk yield by 11.3% was reported after the feed was supplemented with 0.8 kg of concentrate per liter of milk produced (Fike et al. 2003). Besides, many scholars have illustrated an improved daily milk yield from 7 to over 24 liters (Lanyasunya et al. 2001) and 6.7-7.6% (Garg et al. 2014) after cows were fed a balanced ration. The technical feed intervention significantly (P<0.05) improved milk protein by 3.05 and 0.25%, respectively, in medium and large-sized farms, while it decreased fat by 9.89 and 0.56%, in that order (Table 3). The decreased milk fat content in response to supplemented ration is consistent with the study finding reported by Garg et al. (2014). The decreased fat content can be attributed due to an increase in milk quantity in response to the higher concentrate supplementation which is in line with Ponnusmy et al. (2019).

An increased milk yield and milk components in this study could be due to an increased rumen microbial nitrogen synthesis in response to the feed optimization. Besides, the decreased MUN implied that cows were fed on a balanced ration with a suitable protein to energy ratio. The technical feed intervention also significantly (P<0.05) decreased the incidence of reproductive disorders as compared to pre-intervention (Table 4). The

reduction of anestrus incidence is consistent with Alam and Sarder (2010). The more incidence of anestrus in the larger-sized farms might be due to the poor heat detection practices because of their large number of dairy animals. The reduction of placenta retention to zero percent in large-sized farms could be associated with improved feed management. The general variations in the occurrences of reproductive disorders among the studied farm scales were due to the differences in management and genetic blood level of cows kept in each farm. These results are in agreement with previous findings of Tesfaye and Shamble (2013), who reported the variation in the prevalence of major productive disorders in the dairy cows due to the differences in management and breed of the animals as well as environmental factors. Therefore, more extension efforts are required to educate the farmers on optimum feeding protocols as well as proper reproductive management of dairy animals to realise higher milk productivity.

Conclusions

In the study area, the majority of dairy farmers were used feed which is below the point of biological and economic optima. This study revealed improved milk production and reduced reproductive disorders at the different levels of dairy farms through the involvement of professionals with regular technical advice and feed optimization. Therefore, efforts should be undertaken to educate the farmers on the balanced feeding of dairy animals for improved dairy production in the study area.

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References

Adane M, Kaidi R, Hanzen C, England GC (2017) Risk factors of clinical and subclinical endometritis in cattle: a review. Turk J Vet Anim Sci 41: 1-11

Alam MM, Sarder MJ (2010) Effects of nutrition on production and reproduction of dairy cows in Bangladesh. The Bangladesh Veterinarian 27: 8-17

AOAC (2000) Official Methods of Analysis. International, Arlington, Texas, USA

Azage T, Berhanu G, Dirk H, Berhanu B, Yoseph M (2013) Smallholder dairy production and marketing systems in Ethiopia: IPMS experiences and opportunities for market-oriented development. IPMS (Improving Productivity and Market Success) of Ethiopian Farmers Project Working Paper 31. Nairobi: ILRI

Azage T, Tsehay R, Alemu G, Hizkias H (2000) Milk recording and herd registration in Ethiopia. In Proceedings of the 8th Annual Conference of the Ethiopian Society of Animal Production (ESAP), Addis Ababa 90-104

Belay D, Geert PJ (2016) Assessment of feed resources, feeding practices and coping strategies to feed scarcity by smallholder urban dairy producers in Jimma town, Ethiopia. Springerplus 5: 717

Degefa T, Asmamaw D, Reta D (2011) Brucellosis and some reproductive problems of indigenous Arsi cattle in selected Arsi Zone's of Oromia Regional State, Ethiopia. Glob Vet 7: 45-53

Dessalegn G, Berhan T, Gebreyohanes B (2016) Study of productive and reproductive performance of cross breed dairy cattle under smallholder's management system in Bishoftu and Akaki Towns. Int J Agric 6: 913-917

Fike JH, Staples CR, Sollenberger LE, Macoon B, Moore JE (2003) Pasture forages, supplementation rate, and stocking rate effects on dairy cow performance. J Dairy Sci 86: 1268-1281.

Garg MR, Sherasia PL, Phondba BT, Hossain SA (2014) Effect of feeding a balanced ration on milk production, microbial nitrogen supply and methane emissions in field animals. Anim Prod Sci 54: 1657-1661

Gelila AP (2017) Challenges and opportunities of milk production under different urban dairy farm sizes in Ethiopia. Glob J Dairy Farm Milk Prod 5:274-280

Grimard B, Freret S, Chevallier A, Pinto A, Ponsart C, Humblot P(2006) Genetic and environmental factors influencing first service conception rate and late embryonic/foetal mortality in low fertility dairy herds. Anim Reprod Sci 91:31-44. doi: 10.1016/j.anireprosci.2005.03.003.

Jorritsma R, T Wensing, TAM Kruip, PL Vos and JPTM Noordhuizen (2003) Metabolic changes in early lactation and impaired reproductive performance in dairy cows. Vet Res 34: 11-26

Gupta AG, Deopurkar RL (2005) Microbial study of gynecological infection in cattle. Indian J Anim Repord 14:118-119

Hunduma D (2013) Major reproductive disorders of dairy cows in and around Asella town, Central Ethiopia. J *Vet Med* Anim Health 5: 113-117

Khan MH, Manoj K, Pramod S (2016) Reproductive disorders in dairy cattle under semi-intensive system of rearing in North-Eastern India. *Vet World* 9: 512-518

Lanyasunya TP, Mukisira EA, Lokwaleput IK, Siamba DN (2001) Factors limiting optimization of smallholder peri urban dairy herd production in Kenya. Livestock Community and Environment. In: Proceedings of the 10th Conference of the Association of Institutions for Tropical Veterinary Medicine, Copenhagen, Denmark 27-36

Mekonnen HM, Asmamaw K, Courreau JF (2006) Husbandry practices and health in smallholder dairy farms near Addis Ababa, Ethiopia. Prev Vet Med 74: 99-107

Naceur M, Bouallegue M, Frouja S, Ressaissi Y, KaurBrar S, Ben Hamouda M (2012) Effects of environmental factors on milk yield, lactation length and dry period in tunisian holstein cows, milk production an up-to-date overview of animal nutrition, management and health. Available from: DOI: 10.5772/50803

Pandey GS, Voskuil GCJ (2011) Manual on improved feeding of dairy cattle by smallholder farmers. Golden valley agricultural research trust Lusaka, Zambia, 50

Patel, D, Ponnusamy, K and Verma, AP Verma (2020) Reproductive efficiency of dairy animals in different dairy production systems under field conditions. Int J Livest Res 10: 89-96.

Ponnusamy, K, Chakravarty, R and Sohanvir Singh (2019). Extension interventions in coping of farmers against effect of climate change in dairy farming. Indian J Dairy Sci 72: 430-436

Tesfaye D, Shamble A (2013) Reproductive health problems of cows under different management systems in Kombolcha, Noetheast Ehiopia. Adv Biomed Res **7**: 104-108

Van Marle-Köster E, Webb EC (2014) A Perspective on the impact of reproductive technologies on food production in Africa. Adv Exp Med Biol 752: 199-211

Zdunczyk S, Mwaanga ES, Malecki-Tepicht J, Baranski W, Janowski T (2002) Plasma progesterone levels and clinical findings in dairy cows with post-partum anoestrus. Bull Vet Inst Pulawy 46:79-86

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SHORT COMMUNICATION

Combined effect of treatment with intrauterine antimicrobials and GnRH on the conception rate of repeat breeder Frieswal cattle

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Abstract: Repeat breeding in cattle inversely affects the profitability of dairy industry by decreasing conception rate. Selected 18 repeat breeder cows reared under ideal farm conditions and divided them into three groups of six animals each *viz.,* group I, II and III with four primiparous cows and two second parity cows in each group. The cows in group I were treated with intrauterine preparation containing aqueous solution of povidone iodine (5%w/v) and metronidazole (1%w/v) (Utrodin IU), followed by uterine lavage with 120 mL of normal saline and inseminated them in oestrus after skipping the first heat. The animals in group II were treated with an IM injection of buserelin acetate (Gynarich) post AI. The cows in group III were treated with both Utrodin IU and Gynarich post AI. The conception rate observed in group I, II and III were 66.67% (4/6 cows), 66.67% (4/6 cows) and 83.33% (5/6 cows), respectively. The results of the study indicated that combined use of intrauterine preparation containing metronidazole and povidone iodine, along with GnRH was more satisfactory than their individual use in improving the conception rate of repeat breeder Frieswal cattle under farm conditions in Kerala.

Keywords: GnRH analogue, Metronidazole, Povidone iodine, Ideal farm conditions, Kerala

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Repeat breeding is the major cause of decreased profitability of the dairy industry by reducing the conception rate (Ahmadi and Dehghan, 2007) and increasing the treatment cost. Since it is caused by multiple factors, different treatment strategies have been developed for improving the conception rate. One of the best method is intrauterine treatment with antimicrobials, which restores a healthy uterine environment and improves the fertility (Kumar et al. 2014; Mido et al. 2016). As 40.1% of the total causes of repeat breeding in cattle is hormonal insufficiency (Maurer and Echternkamp, 1985), the hormonal therapy is the most effective method to treat repeat breeders (Tiwari et al. 2019). The administration of GnRH or its analogue, post AI can increase the conception rate in repeat breeder cows (Asaduzzaman et al. 2016; Kaim et al. 2003; Lee et al. 1983). A study was conducted to evaluate the combined effect of intrauterine treatment with metronidazole and povidone iodine and IM administration of GnRH post AI on the conception rate of repeat breeder Frieswal cows of different parity, under ideal farm conditions in Kerala.

Eighteen repeat breeder Frieswal cows reared under ideal farm conditions in Kerala were selected and divided them in to three groups of six animals each *viz*., group I, II and III. Each group consists of four primiparous cows and two cows in second parity. They were in good health and condition and vaccinated them against prevalent diseases and let loose for three hours of exercise on alternate days. Dewormed the cows with albendazole @ the dose rate of 10 mg/kg body weight orally. The cows were machine milked twice a day and having a milk yield of 3000-4000 kg/ lactation. They were fed with chopped Hybrid Napier green grass and concentrate feed containing 20% crude protein and required amount of minerals and vitamins manufactured by School of Applied Animal Nutrition and Feed Technology, College of Veterinary and Animal Sciences, Mannuthy as per the standards set by POP (2016).

The cows in group I were treated with intrauterine preparation containing 60 mL aqueous solution of povidone iodine I.P. 5%w/ v (available iodine 0.5%w/v) and metronidazole I.P. (1%w/v) (Utrodin IU Liquid, Nectar Lab Associates, Calicut, Kerala, India), @ the dose rate of 30-60 mL depending on the size and capacity of the uterus. The intrauterine treatment in all animals was followed by 120 mL normal saline lavage to clear out the uterine

Table 1 The conception rate in treatment groups

Treatment	Group I		Group II		Group III	
	No. treated	Conception rate (%)	No. treated	Conception rate (%)	No. treated	Conception rate (%)
Utrodin IU	6	66.67%				
Gynarich			6	66.67%		
Utrodin IU and Gynarich					6	83.33%

secretions and inseminated them 12 hours after the onset of oestrus with Frieswal bull semen by rectovaginal technique after skipping the first heat. The animals in group II were inseminated 12 hours after the onset of oestrus with Frieswal bull semen by rectovaginal technique and given an IM injection of 2.5 mL Gynarich, which contains a GnRH analogue, busereline acetate 4 µg/mL (manufactured by Intas Pharmaceuticals Ltd, India) immediately after the insemination. The cows in group III received both intrauterine treatment with Utrodin IU Liquid and Gynarich injection post AI.

All the experimental animals were examined on 60-75 days after insemination by rectal palpation technique to confirm pregnancy. Conception rate in all the groups were documented and the efficacy of the treatment was evaluated on the basis of post-treatment conception rate.

The results of the present study are given in Table 1. Highest conception rate of 83.33% was observed in group III after treatment with Utrodin IU and Gynarich, whereas the conception rate (66.67%) noticed in both group I and group II were equal in the study. A higher conception rate of 53.33% (Jana, 2010) and 83.33% (Butani et al. 2016) was reported after treatment with 5% povidone iodine and 1% metronidazole in repeat breeder cows. Increase in conception rate by 21.5% (Asaduzzaman et al. 2016), 63.2% (Kaim et al. 2003) and 25% (Lee et al. 1983), was documented in repeat breeder cows after GnRH post AI treatment. The improvement in fertility after Utrodin IU and Gynarich treatment in the present study can be due to two reasons. Firstly, intrauterine treatment with povidone iodine and metronidazole would have restored the healthy uterine environment and improved the fertility by reducing the aerobic and obligately anaerobic bacterial count in the bovine uterus (Bogaard et al.1992; Koujan et al. 1996; Mido et al. 2016).

Secondly, the positive effect of GnRH on improving the ovulation rate (Yaniz et al. 2004), and preventing delayed ovulation (Hamid and Kamruzzaman, 2017). Since the preovulatory surge of LH normally occurs about 6 hours after onset of estrus (Schams et al. 1977), treatment with GnRH at the time of AI may have induced a secondary surge of LH before or after the spontaneous preovulatory surge of LH. This additional LH may be associated with improvement in conception rate. Administration of single dose of GnRH can increase CL formation (Kaim et al. 2003) and rise in plasma progesterone concentration (Mehni et al. 2012) which is due to hypertrophy and hyperplasia of the luteal cells (Kaim et al. 2003).

Conclusions

Based on the present study, it is concluded that combined effect of treatment with intrauterine preparation containing metronidazole and povidone iodine and GnRH was more satisfactory than using them separately in improving the conception rate of repeat breeder Frieswal cattle under ideal farm conditions in kerala.

References

Ahmadi MR, Dehghan SA (2007) Evaluation of the treatment of repeat breeder dairy cows with uterine lavage plus PGF-2 alpha, with and without cephapirin. Turkish J Vet Anim Sci 31: 125-129.

Asaduzzaman KM, Bhuiyan MMU, Rahman MM, Bhattacharjee J (2016) Prevalence of repeat breeding and its effective treatment in cows at selected areas of Bangladesh. Bangladesh J Vet Med 14: 183

Bogaard AE, Hazen MJ, Kriele CP (1992) Rationale for treatment of retained placenta in cows with neomycin and metronidazole. Vet Rec 130: 349-350

Butani MG, Dhami AJ, Shah RG, Sarvaiya NP, Killedar A (2016) Management of repeat breeding in buffaloes under field conditions using hormonal and antibacterial therapies. Buffalo Bulletin 35: 83-91

Hamid SA, Kamruzzaman SM (2017) Effects of GnRH on conception rate at the time of artificial insemination in crossbred dairy cows. Int J Anim Sci Technol 1: 19-34

Jana D (2010) Clinical trial of metricare IU on the treatment of repeat breeding cows. North-East Veterinarian 10: 13-14

Kaim M, Bloch A, Wolfenson D, Braw-Tal R, Rosenberg M, Voet H, Folman Y (2003) Effects of GnRH administered to cows at the onset of estrus on timing of ovulation, endocrine responses, and conception. J Dairy Sci 86: 2012-2021

Koujan A, Eissa HM, Hussein MA, Ayoub MM, Afiefy MM (1996) Therapeutic efficacy of povidone-iodine (Betadine) and dichloroxylenol (Septocid) in Holstein cows affected with endometritis and/or cervicitis. Acta Vet Hung 44: 111–119

Kumar M, Pant SS, Ram R, Kumar S, Gupta PK (2014) Therapeutic efficacy of levofloxacin along with vitamin E for the management of repeat breeding syndrome in cow under field condition. Inter J Vet Sci 3:155-157

Lee CN, Maurice RL, Pennington JA, Hoffman WF (1983) Efficacy of gonadotropin-releasing hormone administered at the time of artificial insemination of heifers and postpartum and repeat breeder dairy cows. Am J Vet Res 44:2160-2163

Maurer RR, Echternkamp SE (1985) Causes and influences of repeat breeding in beef cattle. Beef Research Program Progress Report 2: 49-51

Mehni SB, Shabankareh HK, Kazemi- Bonchenari M, Eghbali M (2012) The comparison of treating Holstein dairy cows with progesterone, CIDR and GnRH after insemination on serum progesterone and pregnancy rates. Reprod Domest Anim 47: 131-134.

Mido S, Murata N, Rawy MS, Kitahara G, Osawa T (2016) Effects of intrauterine infusion of povidone-iodine on endometrial cytology and bacteriology in dairy cows with clinical endometritis. J Vet Med Sci 78: 551–556

POP (2016) Package of Practices Recommendations, Directorate of Entrepreneurship, Kerala Veterinary and Animal Sciences University, Pookode, India. p32-35

Schams D, Shellenberger E, Hoffman B, Karg H (1977) The oestrous cycle of the cow: hormonal parameters and time relationships concerning oestrus, ovulation, and electrical resistance of the vaginal mucus. Acta Endocrinol 86: 180-192

Tiwari I, Shah R, Kaphle K, Gautam M (2019) Treatment approach of different hormonal therapy for repeat breeding dairy animals in Nepal. Arch Vet Sci Med 2: 028-040

Yaniz JL, Murugavel K, Lopez-Gatius F (2004) Recent developments in oestrous synchronization of postpartum dairy cows with and without ovarian disorders. Reprodu Domest Anim 39: 86-93

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SHORT COMMUNICATION

Surveillance of aflatoxin M1 in milk from Navsari, Gujarat area

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Abstract: Aflatoxin M1 (AFM1) is produced during metabolism of Aflatoxin B1 by animal. Aflatoxin is a mutagen and carcinogen and hence possess severe health hazard problem. AFM1 can pass from animal to human via milk. Thus, AFM1 contaminated milk pose a serious threat to public health. Hence, present study deals with detection of AFM1 from cow and buffalo milk. 36 raw samples of cow and buffalo and 9 pasteurized milk samples were collected in each three season i.e., winter, summer and monsoon. Samples were extracted for AFM1 from milk. Dried samples were analyzed for AFM1 using strip ELISA test. Total of 23.81 % and 41.67 % samples from cow and buffalo respectively showed positive for AFM1. Higher numbers of samples were positive in winter followed by monsoon. None of the sample was positive for AFM1 in summer. Thus, present study revealed that there is seasonal effect on presence of AFM1 in milk sample.

Keywords: Aflatoxin M1, ELISA, Milk, Mycotoxin, Navsari

Aflatoxins, a one type of mycotoxins produced mainly by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Creppy, 2002). Various factors like type of substrate, temperature, storage time, storage conditions etc are important and play a role in production of aflatoxin (Stack & Carlson, 2003). The major classes of aflatoxin are aflatoxinB1/B2 and aflatoxin G1/G2. If animal feed is contaminated with Aflatoxin B1, animal will metabolize Aflatoxin B1 to AFM1 (Zinedine et al. 2007) and is able to pass the blood-milk barrier. AFM1 is mutagenic and carcinogenic and can pass to animal milk. Aflatoxin M1 is the only mycotoxin of concern to food safety of milk and dairy products. Moreover, AFM1 is not inactivated by pasteurization or sterilization (Galvano et al. 1996; Jackson and Groopman, 1999). Hence, monitoring of presence of AFM1 is important to prevent health hazard due to AFM1.

Samples were collected from different taluka of Navsari district in sterile bottles and kept into ice box. Analysis was performed within four hours of sample collection. Pasteurized samples were collected from market. AFM1 was extracted as per instruction given in ELISA kit (Abraxis Inc., USA). Aflatoxin M1 was detected using ELISA strip using ELISA test kit. Kit comprised microtiter well which contained colloidal gold labelled antibodies. Sample was added first in microtiter well (after above mention extration), mixed using dropper provided in kit and incubated as per instruction given in kit. After incubation sample was taken from microtiter well and loaded on ELISA strip which contain control and test line. Colloidal gold labelled antibodies and aflatoxin M1 moves on strip by capillary action. In absence of Aflatoxin M1 in the milk sample, colloidal gold labelled antibody occupied test area and produce a visible line of antibody-antigen reaction. Thus formation of two visible lines indicated a negative result. If Aflatoxin M1 is present in the milk sample, it competes with colloidal gold labelled antibody for binding to Aflatoxin M1 conjugate on test line. If a sufficient amount of Aflatoxin M1 is present in the milk sample or extract, it will fill all of the available binding sites, thus preventing attachment of the gold labelled antibody to the immobilized Aflatoxin M1 conjugate and, therefore no line will develop. The control line is not influenced by the presence or absence of Aflatoxin M1 in the milk sample or extract, and therefore, present in all reactions.

For surveillance of aflatoxin M1, 6 unpasteurized milk samples of cow and buffalo each were tested in winter, summer and monsoon season. Three pasteurized samples were also tested in each season. In winter, three cow milk samples out six samples were positive for AFM1, whereas pasteurized sample was negative for AFM1 (Table 1). Thus, 50 % unpasteurized samples were positive during winter season. In buffalo milk sample, out of six samples, five were positive for AFM1 where as two pasteurized samples also showed positive for AFM1 (Table 1). Thus 83.3 % of unpasteurized and 100 % pasteurized samples showed positive of AFM1 (Fig 1). Thus in winter out of total 7 cow milk samples,

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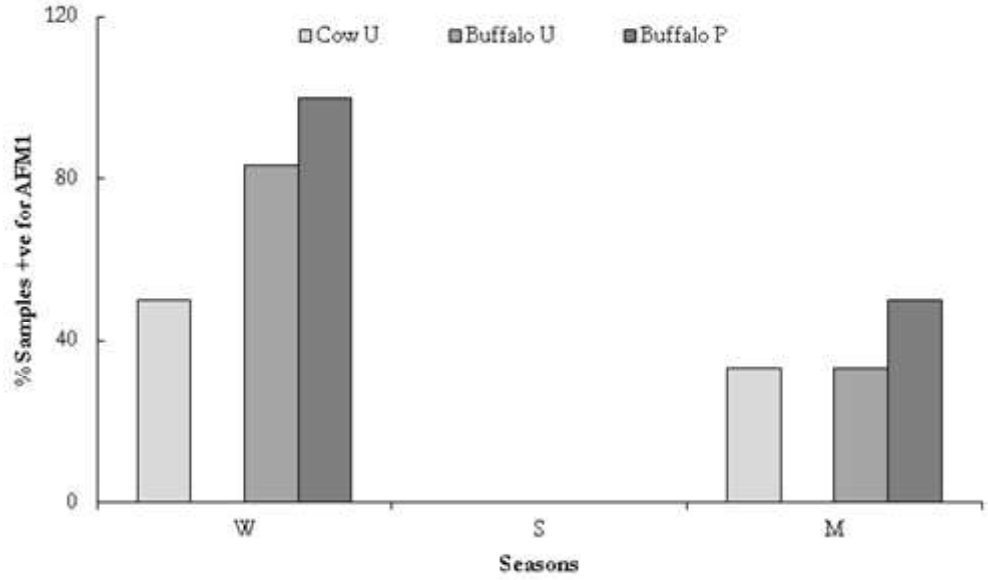

Fig. 1 Seasonal effect on presence of Aflatoxin M1 in cow and buffalo milk samples

3 showed positive for AFM1, whereas, 7 out of 8 buffalo milk samples were positive for AFM1.

During the summer season, none of the pasteurized and unpasteurized cow milk showed positive for AFM1 (Table 1). Similar pattern was also found in buffalo milk samples. None of the sample showed positive for AFM1 in summer (Table 1). In summer, none of showed positive for AFM1out of total 6 and 8 samples of cow and buffalo milk respectively.

In monsoon season, out of six cow milk samples, two were positive, whereas none of the pasteurized milk showed positive for AFM1 (Table 1). Thus, 33.3 % of unpasteurized cow milk samples were positive for AFM1. In buffalo unpasteurized milk samples, two out of six were positive of AFM1 (Table 1). Whereas, in pasteurized buffalo milk samples one was positive for AFM1 (Table 1). Thus, 33.3 % of unpasteurized buffalo milk samples and 50 % pasteurized buffalo milk samples showed positive for AFM1 during monsoon season (Fig 1).

In each season, 6 unpasteurized cow milk sample and 1 pasteurized cow milk was analysed for detection of AFM1. Thus total 21 cow milk samples were analyzed samples in three different seasons. Highest AFM1 positive in raw milk samples were detected in winter season (three) followed by monsoon (two). In summer none of the sample was positive for AFM1.

In all the three season 6 unpasteurized and 2 pasteurized buffalo milk samples were analyzed for AFM1. Thus, total 24 samples were tested for AFM1. Higher AFM1 positive samples were reported in winter followed by monsoon. None of the sample was positive in summer season. Thus, in both cow and buffalo milk samples higher numbers of positive samples were detected in winter followed by monsoon. Data revealed that out of total 45 samples analyzed 27.27 % were positive for AFM1. This ratio for cow and buffalo milk was 23.81 % and 41.67 % for AFM1 positive respectively. Moreover, unpasteurized samples showed higher number of positive compared to pasteurized samples and AFM1 positive samples were more in buffalo milk sample compared cow's milk.

Quality surveillance study was conducted in Navsari area suggested that number of E. coli were higher in winter followed by monsoon an summer (Vyas et al. 2016). Thus, in winter and monsoon season perishable items like milk is more prone to pathogens and toxin.

In one study conducted for mycotoxin detection revealed that 59.3% (n = 64) of milk and cheese samples were detected AFM1, but no sample exceeded the EU legal levels (Batrinou et al. 2020). The levels of AFM1 were found significantly lower in ultra-high temperature pasteurised milk (long-life milk) than in pasteurized milk. In another surveillance of AFM1 in Hisar city of Haryana, India revealed that out of 150 milk samples, 40 samples contained AFM1 below the limit of detection (LOD), 46 raw milk samples contained above LOD and 64 samples showed above limit of quantitation (LOQ) (Sharma et al. 2019). 31 samples showed the AFM1 concentration above 0.5 µg/kg prescribed by FSSAI regulation.

The fungi grow in animal feed and produce aflatoxin B1. Hence, there is great impact of season, storage condition and storage time on mycotoxin production by fungi. There is seasonal effect on AFM1 concentrations in milk samples. Puga-Torres and coworker (2020) reported that 100 % samples were positive for mycotoxin when analyzed by lateral flow immunochromatographic assays. They have reported that all the tested samples were positive for mycotoxin and 59.3 % exceeded the European Union regulatory limit of AFM1. Moreover, they have reported that there is significant difference between season and higher AFM1 positive were in dry season.

Table 1 Presence of aflatoxin M1 in various season in cow and buffalo milk samples

Type of Milk	Pasteurized/Unpasteurized	Total	Winter	Summer	Monsoon
Cow	Total Sample	21	7	7	7
	Total Unpasteurized +Ve	5	3	0	2
	Total Pasteurized +Ve	0	0	0	0
	Total AFM1 +Ve (%)	23.81	-	-	-
	Total Unpasteurized +Ve (%)	-	50	0	33.30
	Total Pasteurized +Ve (%)	-	0	0	0
Buffalo	Total Sample	24	8	8	8
	Total Unpasteurized +Ve	7	5	0	2
	Total Pasteurized +Ve	3	2	0	1
	Total AFM1 +Ve (%)	41.67	-	-	-
	Total Unpasteurized +Ve (%)	-	83.30	0	33.30
	Total Pasteurized +Ve (%)	-	100	0	50

Generally, AFM1 is not inactivated by heat. Thus, pasteurization will not affect the presence of AFM1. However in one research where 85 pasteurised milk samples collected from Ankara, Turkey, were analysed for AFM1 by ELISA method (Celik et al. 2005). They have reported that 88.23 % (75 samples) were contaminated with AFM1. Moreover, 64 % samples were exceeded the legal level as per Turkish Food Codex and Codex Alimentarius limit. Batrinou et al. (2020) reported that AFM1 levels were found significantly lower in ultra-high temperature pasteurised milk (long-life milk) than in pasteurized milk.

Visconti et al. (1985) conducted a surveillance of AFM1 in southern Italy. They have collected raw milk (31 samples), heat-treated milk (66 samples) and dried milk (9 samples). Out of 106 samples tested 76 (72%) samples were positive for AFM1. The AFM1 concentration was in the ranged between 4 to 480 ng/Kg. Higher incidence of AFM1 contamination was reported in commercial milk (91%) than farm milk (26 %). However, the highest AFM1 was reported in dried milk (100 %). One of the reason for high incidence in processed milk was probably the processed feeds used for cattle destined the commercial milk production. One study conducted in Tamilnadu where 45 samples of UHT milk and 52 raw milk samples analyzed were also showed positive of AFM1 (Siddappa et al. 2012). 38% of UTH milk samples contained more than 0.5 µg/kg prescribed limit of Codex Alimentarius Commission and FSSAI Regulations, 2011. Also 61.6% of 52 samples tested showed positive of AFM1 from Karnataka and Tamilnadu area.

In one surveillance of AFM1 conducted by Anand Agricultural Univerisity, Anand reported that 32 out of 38 buffalo milk samples and 30 out of 34 cow milk samples collected around Anand were positive for AFM1 (Choudhary et. al, 1997). AFM1 concentration was 0.076 µg/l and 0.143 µg/l f from buffalo and cow milk samples respectively.

Conclusions

Present study revealed that there is seasonal effect on presence of AFM1 in milk samples. Moreover, there was higher incidence of AFM1 in buffalo milk than in cow's milk samples. However, detail study with higher number of samples will provide more insight on seasonal effect and type of milk i.e., cow or buffalo.

References

Batrinou A, Houhoula D, Papageorgiou E (2020) Rapid detection of mycotoxins on foods and beverages with enzyme linked immunosorbent assay. Quality Assurance Saf Crops Foods 12: 40–49

Çelik TH, Sarimehmetoglu B, Kuplulu O (2005) Aflatoxin M1 contamination in pasteurised milk. Vet Arhiv 75:57-65

Choudhary PL, Sharma RS, Borkhatriya VN, Murthi TN, Wadodkar UR (1997) Survey on the levels of aflatoxin M1 in raw and market milk in and around Anand Town. Indian J Dairy Sci 50: 156-158

Creppy EE (2002) Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol Lett 127: 19-28

Galvano F, Galofaro V, Galvano G (1996) Occurrence and stability of Aflatoxin M1 in milk and milk products: a worldwide review. J Food Prot 59: 1079-1090

Puga-Torres B, Salazar D, Cachiguango M, Cisneros G, Gomez C (2020) Determination of aflatoxin M1 in raw milk from different provinces of Ecuador. Toxins 12: 498

Sharma H, Jadhav VJ, Garg SR (2019) Aflatoxin M1in milk in Hisar city, Haryana, India and risk assessment. Food Add Contam Part B, DOI: 10.1080/19393210.2019.1693434

Siddappa V, Nanjegowda DK, Viswanath P (2012) Occurrence of aflatoxin M-1 in some samples of UHT, raw and pasteurized milk from Indian states of Karnataka and Tamilnadu. Food Chem Toxicol 50:4158-4162

Stack, J, Carlson, M (2003) NF571 *Aspergillus flavus* and aflatoxins in corn, plant diseases, C-18, field crops. Lincoln: Historical Materials from University of Nebraska

Visconti A, Bottalico A, Solfrizzo M (1985) Aflatoxin M1 in milk, in Southern Italy. Mycotoxin Res 1:71–75

Vyas TK, Desai P, Patel A, Patel S, Jajda H, Patel KG (2016) Quality surveillance of milk for microbiological and chemical adulterant sold by local venders at Navsari, India. Trends Life Sci 5: 17-21

Zinedine A, Gonzalez-Osnaya L, Soriano JM, Molto JC, Idrissi L, Manes J (2007) Presence of aflatoxin M1 in pasteurized milk from Morocco. Int J Food Microbiol 114: 25-29

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SHORT COMMUNICATION

Constraint analysis of dairy sector in North Eastern Region: A producers and consumers perspective

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Abstract: Livestock sector act as an alternative source of income for the rural population in the North East Region (NER) of India. But at the same time various limitations hinder the growth of this sector. So, the study was undertaken with the objective to examine the constraints faced by the dairy farmers and consumers of milk and milk products in the region. Assam and Meghalaya were selected purposively as these states were the leading states in cattle population in NER. A random sample of 110 dairy farmers and 174 consumers were selected for collecting primary data on constraints faced by them. The data analyzed using Garret's technique. The major constraints faced by the dairy farmers were the high cost of cattle feed and fodder, inadequate availability of green fodder, lack of organized set up for milk procurement and sale. For the consumers, the major constraints were adulteration, lack of desired quality and inadequate quality control along the value chain. Thus, efforts should be made from the government to take proper steps in regulating the feed prices. Additionally, efforts to set up quality control centre at the block/cluster level so that they can monitor the quality regularly which will further enhance the quality of milk and milk products which would help both the dairy farmers and consumers.

Keywords: Assam, Block, Cluster, Garret's ranking, Meghalaya

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India is the world's largest milk producer producing 184.7 million metric ton (MT) of milk with the per capita availability of 394gm/day (NDDB, 2021).Though the country is self-sufficient in milk production there exist significant regional variation in milk production and in this regard, the North East Region (hereafter NER) is deficit in milk production. During 2018-19, the per capita availability of milk was only 71 gm/day in Assam and 84 gm/day in Meghalaya which are below all India average (NDDB, 2021). The growth of livestock sector has been found to be slower in the NER compared to the national level (Kumar et al. 2007) which may be attributed due to poor resource endowment in terms of number of crossbred, number of cooperatives, available marketing channels and other dairy infrastructures (Feroze *et al,* 2017).

Majority of the population in the NER belongs to small and marginal farmers. So far, the region is concerned the landless and marginal households keep livestock (NSSO, 2003). Livestock sector plays a vital role in economic activities for the landless and marginal households of the region. It also meets the nutritional requirement of the family (Athare et al. 2019).But significant advantages of this sectors from the point of view of demand arise for the livestock rearers from the fact that majority of the population in the NER prefer consumption of meat in their daily diet apart from consumption of milk and milk products.

However, there are number of constraints being faced by both the producers and the consumers. Evaluating the constraints will serve as one of the important tools for the policy making in the developmental activities of the dairy sector. So, the study has been carried out with an objective to evaluate the constraints faced by the dairy farmers and consumers of the NER.

The study was conducted in two states of the NER *viz* Assam and Meghalaya as these are the leading states in cattle population in the NER. From Assam, two districts were selected namely Kamrup (Metro) and Kamrup (Rural), from Meghalaya, Ri-bhoi district was selected. Six blocks were selected from two districts of Assam and one block from Meghalaya was selected. From the selected blocks of Assam, 14 villages were selected at random. From the selected block of Meghalaya, 2 villages were selected at random. A total 284 respondents were considered for the study

of which 110 were dairy farmers and 174 were consumers. Data were collected using well-structured interview schedule.

To find out the constraints faced by the various stakeholders in production and consumption point of view in the study area, Garrett's ranking technique was applied. The constraints were prioritized by using the following formula:

$$Per\ cent\ Position = \frac{100(R_{ij} - 0.05)}{N_j}$$

Where,

R_{ij}= Rank given for the i^{th} item by j^{th} respondent

N_j= Number of items ranked by respondent

The percentage position of each rank was converted into scores using Garrett Table given by Garrette and Woodworth (1969). For each constraint, scores of individual respondents were added together and divided by total number of respondents for whom scores were added. Then, mean score for each constraint was ranked by arranging them in the descending order. Ranks were assigned and most important factors were identified.

Constraints refer to the problems faced by various stakeholders in carrying out various operations and management of various activities. It was observed that high input costs including feed and concentrate (65.44 %) was the major constraints for the dairy farmers in the study area (Table 1). This finding coincides with the findings of (Bhawar et al. 2020; Smitha et al. 2019; Lalrinsangpuii et al. 2016) in different states of India. The dairy farmers reported that during summer months there is shortage of green fodder so they have to compensate through other feed and concentrates, but majority of the farmers could not afford much as the prices of those feed and concentrates were high.

Inadequate availability of green fodder (63.20%) was the second major constraint. This finding correlates with the finding of Rathod et al. (2009) in Karnataka that 91.66 per cent of the respondent reported non-availability of quality feed and fodder round the year. Similar finding was also reported by Smitha et al. (2019) in Kerala and Adhikari et al. (2020) in Uttarakhand. Since majority of the dairy farmers were landless farmers, they did not cultivate fodder crops separately, they depend on the common property resources for grazing animals and also for fodder collection (Feroze et al. 2017), so often there is shortage of green fodder especially during summer months. The dairy farmers in the study area do not grow fodder in their land purposively for livestock purpose. Grasses grown in the open fields and forest and crop residual *viz.,* were used as green fodder and dry fodder for dairy purpose.

Lack of organized set up for milk procurement and sale (61.03%) was also one of the major constraints. This finding coincides with the finding of Lalrinsangpuii et al. (2016) in Mizoram. The respondents from Meghalaya reported that since there were no other co-operatives apart from the existing one so it creates a problem in selling of milk. Most of them were forced to sell their milk in the existing co-operatives since there was not much option for them to dispose off their produce. Some of them further reported that the existing co-operatives were not functioning properly and failed to make the payment on time. About 60.71 per cent of the producer reported decreasing grazing area, long distance to reach the selling point (58.22%), high transportation charges (56.13%), lack of raw milk storage facility (54.91%), occurrence of diseases and parasitic infection (54.07%), non-remunerative price of milk and milk products (50.95%), inadequate veterinary service (50.33%), inadequate veterinary support

Table 1 Constraints faced by the dairy farmers in the NER

Sl. No.	Particulars	Average Score	Rank
1	High input costs including feed and concentrate	65.44	I
2	Inadequate availability of green fodder	63.20	II
3	Lack of organized set up for milk procurement and sale	61.03	III
4	Decreasing grazing area	60.71	IV
5	Long distance to reach the selling point	58.22	V
6	High transportation charges	56.13	VI
7	Lack of raw milk storage facility	54.91	VII
8	Occurrence of diseases and parasitic infection	54.07	VIII
9	Non remunerative price of milk and milk products	50.95	IX
10	Inadequate veterinary service	50.33	X
11	Inadequate veterinary support	49.98	XI
12	Delayed payment by vendors	46.31	XII
13	Lack of labour	41.51	XIII
14	Effect of climatic change including extreme summer and winter	23.23	XIV
15	Interference of local people	20.04	XV

Table 2 Constraints faced by consumer household in the NER

Sl.No.	Particulars	Average Score	Rank
1	Adulteration	66.71	I
2	Lack of desired quality	60.22	II
3	Inadequate quality control	60.21	III
4	Non-availability of desired products	53.78	IV
5	Fluctuating price of milk and milk products (MMPs)	52.72	V
6	Cheating in quantity	46.91	VI
7	Poor packaging	46.07	VII
8	Expiry items with wrong label	44.14	VIII
9	Timely supply	38.05	IX
10	Affordability	29.50	X

(49.98%), delayed payment by vendors (46.31%), lack of labour (41.51%), effect of climatic change including extreme summer and winter (23.23%) and interference of local people (20.04%).

It was observed that majority of the consumers *i.e.,* 66.71 per cent were facing adulteration as the major constraint (Table 2). This may be due to the fact that most of the respondents were taking milk from the milk vendors and reported that the milk was adulterated by adding water.

About 60.22 per cent of the respondents reported that lack of desired quality was the second major constraint. As most of the respondent were availing milk through informal channel *i.e.* vendors, so getting a quality product is a great matter of challenge. About 60.21 per cent of the consumers reported lack of quality control. The consumers reported lacking of quality control right from milk producer level. Further reported, that sometimes the milk gets spoiled easily. This may be due to lack of hygiene during milking and handling process. The non-availability of desired products (53.78%) is also one of the major constraints in the study area. The consumers were willing to get pay for the quality products but they are not getting the quality that they desired for. Fluctuating price of milk and milk products (52.72%) is also one of the major constraints. Lalrinsangpuii et al. (2016) reported the similar finding in Mizoram. Frequent fluctuation in milk price may be due to change in various input cost. Frequent fluctuation in price of milk and its products caused a lot of problems for the consumers as milk is one of the basic necessities which are needed for every day's diet.

Other constraints include cheating in quantity (46.91%), poor packaging (46.07%), out-dated or expiry items with wrong label (44.14%) followed by timely supply (38.05%) and affordability (29.50%).

Conclusions

The study concluded that the high input costs of feed &fodder and adulteration were the major challenges faced by the dairy farmers and the consumers in the NER. So, it is recommended that government must take proper steps in regulating the prices for the feed and concentrate and also an effort to set up quality control centre at the block level so that they can monitor it regularly which will enhance the quality of milk and milk products.

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References

Adhikari B, Chauhan A, Bhardwaj N, Kameswari VLV (2020) Constraints faced by dairy farmers in hill region of Uttarakhand. Indian J Dairy Sci 73(5):464-470

Athare PG, Verma A, Malhotra R, Sendhil R (2019) Economics of milk production in Pune district of Maharashtra: A comparative analysis. Indian J Dairy Science 72: 652-658.

Kumar A, Staal S, Elumalai K, Singh DK (2007) Livestock Sector in North- Eastern Region of India: An Appraisal of Performance. Agric Econ Res Rev 20:255-272

Bhawar RS, Dixit PK, Sivaram, M (2020) Constraints faced by the dairy farmers in production and marketing of milk in northern dry zone of Karnataka. Indian J Dairy Sci 73:274-279.https://doi.org/10.33785/IJDS.2020.v73i03.014

Feroze, SM, Singh, R, Sirohi S (2017) Fodder and labour for augmenting milk production in hills: A case study of Meghalaya. Indian J Dairy Sci 70: 611-615

Garrette, HE, Woodworth, RS (1969) The significance of the difference between means and other statistics. Statistics in Psychology and Education (New York: David Mckay Co. Inc., 1996): 228

Lalrinsangpuii, Malhotra R, Priscilla (2016) Economics of milk production and its constraints in Mizoram. Indian J Dairy Sci 69:588-594

NDDB (2021). NDDB statistics, NDDB, Anand, India. Accessed from https://www.nddb.coop/information/stats/milkprodindia.

NSSO. (2003) Unit level data on land and livestock holdings (59th Round). National Sample Survey Office. Ministry of Statistics and Programme Implementation, Government of India, New Delhi

Rathod PK, Langde S, Nikam TR, Vajreshwari S (2009) Socio-personal profile and constraints of dairy farmers. Karnataka J Agric Sci 24: 619-621

Smitha, S, Devi MCA, Letha DG, Subhas S (2019) Analysis of constraints in dairy farming in Kerala-multistakeholder perspective. Indian J Dairy Sci 72: 342-346. doi.org/10.33785/IJDS.2019.v72i03.016

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SHORT COMMUNICATION

Milk performance of dairy cows supplemented with a combination of slow-release nitrogen and exogenous fibrolytic enzyme

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Abstract: The purpose of this on-farm trial was to evaluate the impact of supplementing slow-release nitrogen (SRN; min. 40% N) and exogenous fibrolytic enzyme (EFE; cellulase, xylanase, glucanase, mannanase and xylanase-potentiating factor) products on milk yield and composition in dairy cows. Twelve early-to-midlactating crossbred cows were divided into 2 groups with comparable milk production. Animals were fed on a total mixed ration comprising of green maize, wheat straw and concentrate mixture. Group T_1 was supplemented with urea at 80 g/animal/d, whereas that of T_2 received a novel combination of cellulo-polymer-coated SRN at 80 g and EFE at 4 g/animal/d for a period of 20 days. Results showed no difference in milk yield (13.8 vs. 15.1 kg/d; P=0.559) between the groups; however, milk fat (3.49 vs. 3.87%) and solid not fat (7.92 vs. 8.35%) levels were higher ($P \leq 0.05$) in group T_2 than T_1. Furthermore, on applying 2-axis milk payment system, group T_2 demonstrated a return on investment of 8.3:1. It was, therefore, concluded that supplementing a combination of SRN and EFE carries practical worth to enhance milk composition, thereby boosting economic gain in dairy farming under Indian context.

Keywords: Farm profitability, Fibrolytic enzyme, Milk production, Nutrient synchrony, Slow-release nitrogen

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Feeding a balanced diet adequate in all essential nutrients is the cornerstone of productive performance of dairy animals. Protein sources are considered as the most expensive ingredient components in dairy rations. However, ruminants can utilise non-protein nitrogen (NPN) in presence of available fermentable energy to synthesise rumen microbial crude protein (MCP; AFRC, 1993; NRC, 2001). The MCP is of high nutritional significance, given the fact that it could meet as much as 70-100% of protein requirements of cows depending on the level of productivity (Thirumalesh and Krishnamoorthy, 2013). While urea is the most commonly employed NPN source, due to its instant solubility and ammonia (NH_3) release in the rumen, it becomes a real challenge to achieving optimum synchrony with that of carbohydrate source to maximise MCP production (Inostroza et al. 2010; Salami et al. 2021). Consequently, NH_3 that is not captured by microbial cells would get absorbed across ruminal wall and excreted (wasted) through urine. On the other hand, if urea is made to deaminate slowly—thus controlling NH_3 release over a sustained period of time in the rumen—it could theoretically enhance the yield of MCP (AFRC, 1993; NRC, 2001; Cherdthong and Wanapat, 2010), minimise urinary N losses and safeguard the health of dairy cows from possible adverse effects of urea (Salami et al. 2021). It is interesting that on 'protein equivalent' basis, 1 unit of slow-release N (SRN) could equate to 5-7 kg of traditional oilseed cakes/meals, and hence appears to lessen the feed cost.

Although rumen represents a fountain of enzyme systems, there seems a limitation for the digestion of lignocellulosic fibre fractions, owing to the highly recalcitrant structure of forage cell walls (Mahesh and Mohini, 2013). Hence, researchers have explored the possibility to improve fibre utilisation and subsequent performance with the use of exogenous fibrolytic enzymes (EFE; Gado et al. 2009; Shekhar et al. 2010; Meale et al. 2014) under diverse dietary and productivity scenarios (Tirado-González et al. 2018).

Considering the above points, it was our general hypothesis that supplementing novel feed additives influencing protein and energy metabolism could boost production, whilst also impacting bottom-line farm profits. The objective of the present experiment

was to assess the response of dairy cows supplemented with a combination of SRN and EFE under field conditions.

This trial was performed at a commercial dairy farm located in north India. Twelve crossbred cows in their early-to-midlactation stage were selected and stratified into 2 groups (T_1 and T_2) of 6 each in such a way that both had similar milk yield before commencement of the trial. All the animals were fed a total mixed ration comprising of specified proportion of chopped green maize (18.3% dry matter, 8.2% crude protein and 42% neutral detergent fibre), concentrate mixture (20.8% crude protein, 4.2% ether extract and 10.8 MJ of metabolisable energy/kg) and wheat straw to meet/exceed the nutrient requirements (ICAR, 2013). Cows in group T_1 were supplemented with technical grade urea at 80 g/animal/day, while that of T_2 received a novel combination of cellulo-polymer-coated SRN (Zenitro™ with min. 40% N) at 80 g and EFE (Kemzyme® containing cellulase, xylanase, glucanase, mannanase and xylanase-potentiating factor) at 4 g/animal/day for a period of 20 days (Table 1). Both the additives were manufactured by Kemin Industries South Asia Pvt. Ltd., Chennai, India. Animals were hand-milked twice a day and quantity of milk produced was recorded daily on individual basis. Similarly, milk composition in terms of fat and solid not fat (SNF) was analysed by EKOMILK analyser (BULTEH 2000 Ltd, Bulgaria). Ad libitum fresh drinking water was made available for all cows throughout the day. Statistical analysis was carried out using Statgraphics Centurion XVI software (version 16.2.04). The data were analysed by t-test and a P-value ≤ 0.05 was considered statistically significant.

Results showed that milk yield did not differ between cows of groups T_1 and T_2 (13.8 vs. 15.1 kg/d; P=0.559; Table 2). However, composition of milk expressed as fat and SNF improved (P≤0.05) in group T_2 than that of T_1. Furthermore, the economic benefit measured as return on investment (ROI) was found to be 8.3:1 in group T_2 (Table 2).

A positive milk response to dietary supplementation is expected when one or many nutrients are deficient in the basal diet, and both the diets fed in the present experiment were similar in all nutrient profile, matrix values and even had similar DMI (~15 kg/d; data not shown). This might explain the absence of any effect of supplementation on milk yield.

Despite the diets were balanced to match the current production levels, it is interesting to observe an improvement in milk fat and SNF by 10.9% and 5.4%, respectively in group T_2. This can be justified by the individual role of SRN in improving rumen N metabolism (AFRC, 1993; Cherdthong and Wanapat, 2010; Mahesh et al. 2017; Salami et al. 2021) and EFE in augmenting fibre digestion (Shekhar et al. 2010) – put together resulting in higher availability of nutrient precursors such as acetate for milk fat (Inostroza et al. 2010) and MCP for milk protein synthesis (Thirumalesh and Krishnamoorthy, 2013). On quantitative terms, it appears that MCP fulfilled approximately 78% of total protein requirements (1.4 kg MCP vs. 1.8 kg protein requirement/day; ICAR, 2013) assuming a theoretical MCP yield of 170 g/kg organic matter fermented in the rumen (DOMR; Blümmel et al. 1999). Indeed, the level of both the NPN sources used were 80 g representing not >15% of total N intake, which is within the safer dietary inclusion level of 135 g and/or 20% of total daily N intake recommended for dairy cows (Kertz et al. 2010). Furthermore, it is also reasonable that, along with sustained release effect of NH_3 by SRN, the fibre degradation carried out by EFE might have additionally supported the availability of fermentable carbohydrates, as has been reported previously (Gado et al. 2009; Shekhar et al. 2010; Meale et al. 2014). In this direction, a recent meta-analysis by Tirado-González et al. (2018) conclusively revealed an increase of 99.4 g milk protein and 83 g fat per day upon supplementing EFE in dairy cows, as obtained for group T_2 in the present experiment. According to the best of authors'

Table 1 Ingredient composition of total mixed ration (kg, as-is basis)

Ingredient	T_1	T_2
Green maize	30	30
Wheat straw	3	3
Concentrate mixture[a]	8	8
Urea	0.08	-
Slow-release nitrogen	-	0.08
Exogenous fibrolytic enzyme	-	0.004

[a]Inclusive of micronutrients and additives

Table 2 Production performance and economics of novel additive supplementation in dairy cows (n=6)

Attribute	T_1	T_2
Production		
Milk yield (kg/d)	13.8 ± 1.37	15.1 ± 1.48
Fat (%)	3.49^{A} ± 0.06	3.87^{B} ± 0.08
Solid not fat (%)	7.92^{A} ± 0.04	8.35^{B} ± 0.02
Economics		
Price of milk (INR/kg)[a]	30	33
Milk revenue (INR/d)	414	500
Incremental revenue (INR/animal/d)	-	86
Inclusion cost of additives (INR/d)	-	10.4
Return on investment[b]	-	8.3

1$ = INR 74

[a]Based on prevailing 2-axis farm-gate milk pricing system in the market

[b]Incremental revenue due to treatment ÷ additional cost of supplementation

Means bearing different superscripts (A, B) in each row differ significantly at P≤0.05

knowledge, this is the first-of-its-kind of study demonstrating the practical worth of a combination of SRN and EFE to benefit milk components in dairy cows under Indian context.

A good 'economic return' is a central determinant of sustainability of any dairy enterprise. As feed alone is factored towards >60% of recurring expenses, any dietary strategy is expected to have a direct impact on farm economy. Considering fat and SNF-based 2-axis milk payment system, the outcome of present trial clearly deduces that an ROI of 8.3:1 is practically achievable when dairy producers use a novel combination of SRN and EFE for lactating rations. This is principally attributed to an increased farm-gate milk realisation as a result of improved milk composition. Consistent with our findings, when SRN partly substituted soya bean meal, a better income over feed cost was observed in high producing Wisconsin dairy herds (Inostroza et al. 2010).

Conclusions

It was concluded from this on-farm trial that dairy cows supplemented with a combination of slow-release nitrogen and exogenous fibrolytic enzyme improved milk composition and net economic returns. Future studies may explore the trade-offs between feed cost optimisation with these additives through energy-protein ingredient reformulation and performance measures in dairy cows.

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Conflicts of interest

Authors declare that there are no known financial or any other conflicts of interest associated with the data presented in this publication.

References

AFRC (1993) Energy and protein requirements of ruminants. Agricultural and Food Research Council, CAB International, Wallingford, UK

Blümmel M, Mgomezulu R, Chen XB, Makkar HPS, Becker K, Ørskov ER (1999) The modification of an in vitro gas production test to detect roughage related differences in in vivo microbial protein synthesis as estimated by the excretion of purine derivatives. J Agric Sci (Cambridge) 133: 335-340

Cherdthong A, Wanapapt M (2010) Development of urea products as rumen slow-release feed for ruminant production: a review. Aust J Basic Appl Sci 4: 2232-2241

Gado HM, Salem AZM, Robinson PH, Hassan M (2009) Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Anim Feed Sci Technol 154: 36-46

ICAR (2013) Nutrient Requirements of Cattle and Buffalo. Indian Council of Agricultural Research, New Delhi, India

Inostroza JF, Shaver RD, Cabrera VE, Tricárico JM (2010) Effect of diets containing a controlled-release urea product on milk yield, milk composition, and milk component yields in commercial Wisconsin dairy herds and economic implications. Prof Anim Sci 26: 175-180

Kertz AF (2010) Urea feeding to dairy cattle: a historical perspective and review. Prof Anim Sci 26: 257-272

Mahesh MS, Mohini M (2013) Biological treatment of crop residues for ruminant feeding: a review. Afr J Biotechnol 12: 4221-4231

Mahesh MS, Thakur SS, Kumar R, Malik T, Gami R (2017) Nitrogen fractionation of certain conventional- and lesser-known by-products for ruminants. Anim Nutr 3: 186-190

Meale SJ, Beauchemin KA, Hristov AN, Chaves AV, McAllister TA (2014) Opportunities and challenges in using exogenous enzymes to improve ruminant production. J Anim Sci 92: 427-442

NRC (2001) Nutrient requirements of dairy cattle. 7th rev. edn. National Academy Press, Washington, DC, USA

Salami SA, Moran CA, Warren HE, Taylor-Pickard J (2021) Meta-analysis and sustainability of feeding slow-release urea in dairy production. PLoS ONE 16: e0246922

Shekhar C, Thakur SS, Shelke SK (2010) Effect of exogenous fibrolytic enzymes supplementation on milk production and nutrient utilization in Murrah buffaloes. Trop Anim Health Prod 42: 1465-1470

Thirumalesh T, Krishnamoorthy U (2013) Rumen microbial biomass synthesis and its importance in ruminant production. Int J Livest Res 3: 5-26

Tirado-González DN, Miranda-Romero LA, Ruíz-Flores A, Medina-Cuéllar SE, Ramírez-Valverde R, Tirado-Estrada G (2018) Meta-analysis: effects of exogenous fibrolytic enzymes in ruminant diets. J Appl Anim Res 46: 771-783