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RESEARCH ARTICLE

Influence of in-package microwave treatment and geometry on selected characteristics of *Paneer*

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Abstract: In this paper, the influence of in-package microwave treatment and geometry (cylinder and cube) of *paneer* on its colour, texture and sensory characteristics was investigated. The freshly prepared *paneer* was moulded into cylinder and cube shapes with the help of moulds and packed in polypropylene pack. This packed *paneer* was exposed to different level of microwave power (270, 360 and 450W) for varying time (10, 20 and 30s) and evaluated for colour, texture and sensory attributes of the *paneer*. It was observed that microwave power and exposure time had significant effect on colour, texture and sensory quality of both cylinder and cube shaped *paneer*. It was observed that all important quality and properties of *paneer* viz. whiteness index, hardness, flavour and overall acceptability were higher in cube shaped *paneer* than that of cylinder shaped *paneer*. Therefore, it may be recommended that for in-package microwave treatment, a cube shaped *paneer* may be used over cylindrical shape.

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Introduction

Paneer, a south-Asian variety of soft cheese, is obtained by acid and heat coagulation of milk. It is popular throughout south-Asia and used in raw form or in preparation of several varieties of culinary dishes and snacks. Day by day, the production of *paneer* is spreading throughout the world. The ability of *paneer* to be deep fried is one of the important features that have led to its wider acceptance and a favourite for making snacks, such as *pakoras* or fried *paneer chunks* (Aneja, 2007). Good quality *paneer* is characterized by a marble white colour, sweetish, mildly acidic taste, nutty flavour, spongy body and closely knit smooth texture. It is a rich source of protein available at a comparatively lower cost and forms an important source of animal protein for vegetarians. In addition to its high protein content and digestibility, the biological value of protein in *paneer* is in the range of 80 to 86 (Shrivastava and Goyal, 2007).

Paneer is a valuable source of fat, vitamins and minerals like calcium and phosphorus. It has a reasonably long shelf life under refrigeration. The production of *paneer* has been largely confined to the unorganised dairy sector which employs traditional, inefficient methods of manufacture. Due to the ever growing demand for *paneer*, researchers were encouraged to develop new techniques for the manufacture of *paneer*. Researchers recommended varied processing conditions for the preparation of varieties of *paneer* using different types of milk.

Microwaves are electromagnetic waves whose frequency and wavelength varies from 300 MHz to 300 GHz and 1mm to 1m, respectively. Generally, domestic microwave appliances operate at a frequency of 2.45 GHz, whereas industrial microwave systems operate at frequencies of 915 MHz and 2.45 GHz (Giese, 1992). Microwave heating is caused by the ability of the materials to absorb microwave energy and convert it into heat. Microwave heating of food materials mainly occurs due to dipolar and ionic mechanisms. There are many factors which affect the microwave heating and its heat distribution and the most important of them are the dielectric properties and penetration depth.

There are various applications of microwaves in dairy industry e.g. microwave heating of yoghurt (Turgut, 2016), microwave drying of *paneer* (Singh and Rai, 2004), cultured milk products (Sarkar, 2006), milk pasteurization (Kumar, 2009), continuous microwave processing of *paneer* (Karthikeyan et al. 2011), microwave stretching of mozzarella cheese (Kishor et al. 2015) and soya fortified *paneer* (Uprit and Mishra, 2004) etc. Badola et al. (2017) studies the combined effect of herbal essential oil and in-package microwave thermization treatment on shelf life of burfi and reported that microwave power level of 10% and treatment exposure time of 60s had statistically significant ($p<0.05$) effect on microbial counts together with non-significant ($p>0.05$) effect on sensory scores.

The flavour and colour of a food product have significant impacts on consumer

acceptability and two of the challenges with microwave food products are (i) it is often difficult to achieve the desired flavour that matches products prepared in a conventional oven or by frying and (ii) to get the browning that the consumer expects. There are some reactions that do not occur when foods are heated in the microwave oven and this is part of what contributes to the lack of flavour and colour development. The typical browning which occurs when foods are heated by conventional means produces not only the desired brown pigments but also produces a variety of desirable flavours.

The effects of geometries (size and shape) of food materials on microwave heating have been reported by some researchers in published literature (Vilayannur et al. 1998; Basak et al. 2014; Hossan et al. 2010; Zhang et al. 2018). The physical size and shape of foods affect the temperature distribution within the food. Vilayannur et al. (1998) studied temperature and moisture distributions in different geometries i.e. brick-shaped, cylinder-shaped and hexagonal prism-shaped products. Hossan et al. (2010) analysed microwave heating for cylindrical shaped objects. The temperature distributions within the body were represented as a function of cylinder length, radius, heat transfer coefficient, and microwave frequency. Basak et al. (2014) analysed the effect of shapes for microwave-assisted food processing of 2D samples. Zhang et al. (2018) studied numerical simulation of shape effect, which was assessed by microwave power absorption capability and temperature distribution uniformity in a single sample heated in a domestic microwave oven. In microwave processing, various shapes e.g. sphere, cube, cylinder, ellipsoid, and cuboid of potato samples on microwave power absorption capability and temperature distribution uniformity before the internal hot spots reach the phase transition temperature were investigated (Zhang et al. 2018).

Therefore, in view of the above, an effort was made to investigate the influence of in-package microwave treatment and geometry (cylinder and cube) of *paneer* on its colour, texture and sensory characteristics.

Materials and Methods

Fresh pooled buffalo milk was received from the Experimental Dairy, ICAR-National Dairy Research Institute, Karnal, Haryana, India. Two type of moulds (cylindrical- 2.1 cm dia × 3 cm height; cubic - 2.1 cm side) were used for moulding the *paneer* into cylindrical and cubical shapes. Stainless steel was used in fabrication of the moulds. Both moulds provide constant mass (11g). Polypropylene microwavable safe packaging materials were procured from M/s Krishna Plastic and Packaging Material, New Delhi. A convection microwave oven (make - IFB; model- 30FRC2; power supply - 230V~50Hz; rated microwave output - 900W; operation frequency - 2450MHz; oven capacity – 30 Litres and cooking uniformity -turntable system) was used for in-package microwave treatment.

Preparation of *Paneer* sample

Paneer was prepared using standard batch method of manufacture (Aneja, 2007) as shown in Fig.1. The prepared paneer was cut into desired shape using mould, immersed in chilled water (4ºC) and packaged in selected packaging material and storage (4±1ºC).

Freshly prepared *Paneer* was moulded with the help of stainless steel (SS) cylinder/cube mould into cylinder / cube shapes having constant mass 11g. It was then packed in polypropylene pack (410 µm) under atmospheric condition and subjected to in-package microwave treatment.

Selection of microwave power level and exposure time for in-packaged *Paneer*

The microwave power level and exposure time was selected based on preliminary trials for the in-packaged *paneer* treatment. The microwave treatment involved subjecting the in-packaged product to three microwave power levels (270, 360 and 450W) and exposure times (10, 20 and 30s) in the convection microwave oven.

Colour Measurement

Whole *paneer* (cubic or cylindrical form) was shredded with the help of a grater (mesh size of grater) and analysed for colour measurement. Colour values (L^*, a^*, b^*) of the *Paneer* samples were determined by using a Colour Measuring System (Labscan, XE, Hunter Lab. Inc., USA). Before colour measurement, the instrument was calibrated with a standard black glass and white glass tiles as specified by the manufactures (Barnwal et al. 2014). Data were obtained from software in terms of L^* {lightness, range: 0 (black) to 100 (white)}, a^* {redness; range: +60 (red) to -60 (green)} and b^* {yellowness, range: +60 (yellow) to -60 (blue)}.

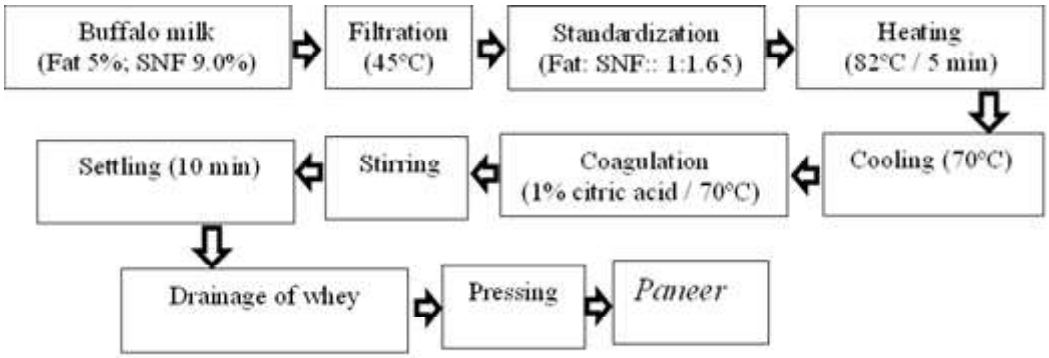

Fig.1 Process flow chart for the manufacture of *Paneer*

Whiteness index (*WI)* of the *Paneer* samples was calculated by using following standard relation (Srinivasa et al. 2017).

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (1)$$

The chroma value, hue angle and browning index (BI) were calculated by using standard relations (Barnwal et al. 2015; Gupta et al. 2011) and used to describe the color change due to shape and in-package microwave treatment of *paneer* samples:

$$Chroma = \sqrt{\left(a^{*2} + b^{*2}\right)} \quad (2)$$

$$Hue\ \ angle = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

$$BI = \left[\frac{100 \times (x - 0.31)}{0.17}\right] \quad (4)$$

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

Sensory evaluation

The 9-point Hedonic scale (9 for 'liked extremely' and 1 for 'disliked extremely') was used for the determination of sensory attributes of the in-packaged microwave treated *Paneer* by discriminative and communicative judges selected based on the discriminative test of sensory evaluation. They evaluated the product for various sensory parameters e.g. colour and appearance, body and texture, flavour and overall acceptability.

Texture Profile Analysis (TPA)

Textural attributes of *Paneer* samples were determined by using a texture analyser (TA-XT2i, Stable Micro System Ltd., Surrey, England) equipped with a 25 kg load cell and calibrated with 5kg standard dead weight (Meena et al. 2014). Compression probe (P-75) was used to compress the whole sample (cube/cylinder) up to 80 percent of its original height (80 percent of its strain), using a double compression test. Before starting the test, the probe (P-75) was calibrated to a distance of 50 mm. The constant probe speed (pre-test speed: 2 mm/sec; speed during test: 1mm/sec; post-test speed: 2mm/sec) were used throughout the study. Sample (cylinder: 2.1 cm dia × 3 cm height, cube: 2.1 cm side) was placed on the platform. Probe was positioned centrally over the sample surface and allowed to compress the product. All the tests were conducted at about temperature of sample maintained at 25±1°C. The force distance compression curve was obtained to estimate the hardness, springiness, cohesiveness, gumminess, chewiness and adhesiveness.

Statistical analysis

The data collected during experimental trials is expressed as mean ± standard deviation for three independent samples, calculated using Microsoft excel software. The data obtained during the present investigation is subjected to the one way analysis of variance (ANOVA) using SPSS version 23 software of M/s IBM Corporation.

Results and Discussion

The one way analysis of variance (ANOVA) for different selected characteristics i.e. colour, texture and sensory characteristics of in-package microwave treated geometry (cylinder and cube) of *paneer* samples are presented in Tables 1-4.

Colour attributes of the in-package microwave treated *Paneer*

Table 1 represents the colour attributes (*L*, a*, b*)* of in-package microwave treated *paneer* (cylinder and cube geometries) under different microwave power and exposure time. For cylindrical shaped *paneer*, *L*, a** and *b** these attributes are highly significant ($p<0.001$) with microwave power, exposure time and their interactions (Table 1).

For cylindrical *paneer* sample, *L**-value ranged from 79.23±0.39 (270W, 20s) to 80.43±0.14 (450W, 10s) whereas for cubical *paneer* sample, it ranged from 80.88±0.46 (450W, 10s) to 82.13±0.10 (270W, 10s). In case of cylindrical *paneer* sample, *a**-value ranged from

-2.60±0.06 (270W, 30 s) to -2.32±0.09 (450W, 20s) and for cubical *paneer* sample, it ranged from -2.49±0.40 (270W, 30s) to -2.06±0.07 (270W, 10 s). The *a**-value was statistically similar for all microwave power at 20 sec microwave exposer time for both shape.

For cylindrical shaped *paneer* samples, the maximum and minimum values of *b**-value were 13.12±0.34 (270W, 30s) and 11.58±0.06 (450W, 20s), respectively. The *b**-value for cubical shaped *paneer* sample ranged from 8.81±0.09 (360W, 20s) to 10.34±0.43 (270W, 30s.) The *b**-value was statistically different for all microwave power at 20 sec.

Colour derivatives of the in-package microwave treated *Paneer*

Colour derivatives (whiteness index, *WI*; Chroma; hue angle, brownness index, *BI*) of in-package microwave treated *paneer* (cylindrical and cubic geometry) are presented in Table 2 under different microwave power and different microwave exposure time. These colour derivatives was highly significant ($p<0.001$) with microwave power (Table 2). The whiteness index of *paneer* (cylinder shape) was ranged from 75.39±0.07 (360W, 20 s) to 76.78±0.13 (450W, 10 s) and for the *paneer* (cube shape); it ranged from 78.14±0.15 (270W, 30s) to 78.97±0.05 (450W, 30s). So it was observed that the whiteness index was more in cube shaped *paneer* than cylindrical (Campanone and Zaritzky, 2005). It was investigated that for cube shaped sample most of the microwave energy was concentrated at the centre. The chroma value of both shaped *paneer* was significant ($p<0.001$) with microwave power and microwave exposure time. Chroma value was maximum and minimum as 13.37±0.03 and 11.81±0.37, respectively for cylinder shaped *paneer,* whereas it was maximum and minimum as 10.65±0.32 and 9.10±0.11, respectively for cube shaped *paneer*.

The parameter hue angle presented negative sign denotes that the perceived colour was less bluish/more yellowish than inherent colour, positive sign denote opposite. *The* hue angle value of (cylinder) shaped and (cube) was almost similar for all microwave

Table 1 ANOVA for Colour attributes of different geometry and in-package microwave treated *Paneer*

Microwave Power (W) ↓	Exposure Time (s) → / Geometry	10	20	30
			Colour attributes	
			*L**	
270	*Cylinder*	80.02±0.01^{aB}	79.23±0.39^{bB}	79.91±0.18^{aA}
	Cube	82.13±0.10^{aA}	81.39±0.05^{bA}	80.92±0.01^{cB}
360	*Cylinder*	80.06±0.03^{aB}	80.08±0.09^{aA}	80.16±0.16^{aA}
	Cube	81.72±0.17^{bA}	82.13±0.02^{aA}	81.46±0.02^{bA}
450	*Cylinder*	80.43±0.14^{aA}	79.94±0.07^{bA}	80.21±0.07^{aA}
	Cube	80.88±0.46^{aB}	81.01±1.13^{aA}	81.40±0.10^{aA}
			*a**	
270	*Cylinder*	-2.58±0.00^{aC}	-2.55±0.04^{aB}	-2.60±0.06^{aB}
	Cube	-2.06±0.07^{aA}	-2.42±0.03^{aA}	-2.49±0.40^{aA}
360	*Cylinder*	-2.43±0.02^{aB}	-2.34±0.08^{aA}	-2.37±0.03^{aA}
	Cube	-2.30±0.06^{aB}	2.28±0.01^{aA}	-2.30±0.01^{aA}
450	*Cylinder*	-2.38±0.01^{abA}	-2.32±0.09^{aA}	-2.49±0.05^{bAB}
	Cube	-2.20±0.10^{aAB}	-2.46±0.10^{bA}	-2.33±0.05^{abA}
			*b**	
270	*Cylinder*	12.52±0.14^{bA}	12.76±0.29^{abA}	13.12±0.34^{aA}
	Cube	9.50±0.11^{bA}	9.64±0.05^{bA}	10.34±0.43^{aA}
360	*Cylinder*	12.79±0.05^{aA}	12.45±0.03^{aA}	12.46±0.17^{aB}
	Cube	9.06±0.07^{bB}	8.81±0.09^{cB}	9.66±0.11^{aB}
450	*Cylinder*	12.26±0.07^{aA}	11.58±0.06^{bB}	12.19±0.37^{aC}
	Cube	9.63±0.18^{aA}	9.49±0.03^{aA}	9.52±0.08^{aB}
F- value		Power	Time	Power × Time
*L**	*Cylinder*	19.52***	15.66***	5.12**
	Cube	5.72*	1.43^{NS}	3.99**
*a**	*Cylinder*	36.04***	5.87**	2.33^{NS}
	Cube	0.13^{NS}	4.49*	2.33^{NS}
*b**	*Cylinder*	24.87***	4.52*	4.53**
	Cube	30.77***	23.18***	7.63***

Note: *Data presented as Mean ± SD values ; ^{abc}Means within column (microwave power) and ^{ABC}Means within a row (exposure time); the same superscript is not significantly different; NS non-significant; *p<0.05; **p<0.01; ***p<0.001; n=3*

power and time. There was non-significant effect observed in both power and time. The *BI* value was statistically same for all microwave power at 10 sec.

BI value was ranged from 13.09±0.42 (450W, 20s) to 15.04±0.06 (270W, 30s) for cylinder shaped *paneer* and in case of cube shaped *paneer*, it was ranged from 9.01±0.03 (360W, 20s) to 11.04±0.97 (270W, 30s). The *BI* value higher in cylinder shaped *paneer* than cube.

It was shown that, under different microwave power and microwave exposure time, the Textural characteristics hardness, springiness, cohesiveness, gumminess, chewiness and resilience of *in-packaged microwave treated paneer* were presented in Table 3. The hardness, cohesiveness, gumminess, springiness and resilience properties were significant ($p<0.05$) with reference to power values of microwave treatments, where the properties like chewiness, cohesiveness, springiness and resilience were non-significant with time for cylinder shaped *paneer*. In case of

Table 2 ANOVA for Colour derivatives of different geometry and in-package microwave treated *Paneer*

Exposure Time (s) ➡		10	20	30
Microwave Power (W) ⬇	Geometry		Colour derivatives	
			Whiteness Index (WI)	
270	*Cylinder*	76.26±0.07^{aB}	75.49±0.44^{bB}	75.86±0.08^{abB}
	Cube	79.65±0.08^{aA}	78.90±0.04^{bA}	78.14±0.15^{cB}
360	*Cylinder*	76.18±0.23^{aB}	75.39±0.07^{aA}	75.45±0.05^{aA}
	Cube	79.47±0.11^{bA}	78.94±0.07^{aA}	78.96±0.18^{cA}
450	*Cylinder*	76.78±0.13^{aA}	76.71±0.19^{aA}	76.62±0.03^{aA}
	Cube	78.48±0.37^{aB}	78.62±1.00^{aA}	78.97±0.05^{aA}
			Chroma	
270	*Cylinder*	12.78±0.14^{bA}	13.01±0.28^{abA}	13.37±0.03^{aA}
	Cube	9.72±0.12^{bA}	9.93±0.06^{bA}	10.65±0.32^{aA}
360	*Cylinder*	13.01±0.49^{aA}	12.67±0.01^{aA}	12.69±0.16^{aB}
	Cube	9.35±0.08^{bB}	9.10±0.11^{bB}	9.99±0.11^{aB}
450	*Cylinder*	12.49±0.07^{aA}	11.81±0.37^{bB}	12.44±0.06^{aB}
	Cube	9.88±0.18^{aA}	9.81±0.05^{aA}	9.80±0.09^{aB}
			Hue angle	
270	*Cylinder*	-1.36±0.00^{aA}	-1.37±0.00^{aA}	-1.37±0.00^{aAB}
	Cube	-1.35±0.00^{aB}	-1.32±0.00^{aA}	-1.33±0.04^{aA}
360	*Cylinder*	-1.38±0.00^{aB}	-1.38±0.00^{aA}	-1.38±0.00^{aB}
	Cube	-1.32±0.00^{abA}	-1.31±0.00^{aA}	-1.33±0.00^{bA}
450	*Cylinder*	-1.37±0.0^{bAB}	-1.37±0.00^{abA}	-1.36±0.00^{aA}
	Cube	-1.34±0.01^{bB}	-1.31±0.00^{aA}	-1.33±0.00^{abA}
			Brownness Index (BI)	
270	*Cylinder*	14.17±0.21^{bA}	14.70±0.51^{abA}	15.04±0.06^{aA}
	Cube	10.13±0.09^{aA}	10.08±0.04^{aA}	11.04±0.97^{aA}
360	*Cylinder*	14.69±0.72^{aA}	14.29±0.11^{aA}	14.26±0.22^{aB}
	Cube	09.36±0.06^{bB}	9.01±0.03^{cB}	10.24±0.15^{aA}
450	*Cylinder*	13.91±0.09^{aA}	13.09±0.42^{bB}	13.58±0.03^{aC}
	Cube	10.34±0.25^{aA}	9.89±0.23^{aA}	10.00±0.05^{aA}
F- value		Power	Time	Power × Time
WI	*Cylinder*	39.51***	2.65^{NS}	5.71**
	Cube	10.31***	5.15*	6.98***
Chromo	*Cylinder*	26.19***	4.89*	4.58**
	Cube	40.55***	31.88***	11.25***
Hue angle	*Cylinder*	14.35***	0.23^{NS}	2.44^{NS}
	Cube	1.36^{NS}	3.87*	1.21^{NS}
BI	*Cylinder*	22.86***	2.00^{NS}	4.43**
	Cube	14.60***	10.52***	3.46*

Note: *Data presented as Mean ± SD values (n=3).* ^{abc}*Means within column (power) and* ^{ABC}*Means within a row (time) the same superscript is not significantly different, NS non-significant, *p<0.05, **p<0.01, ***p<0.001; n=3*

Table 3 One way ANOVA for textural properties of Microwave in-package treated *Paneer* at different Power and Time

Exposure Time (s) →		10	20	30
Microwave Power (W) ↓	Geometry		Textural properties	
			Hardness	
270	*Cylinder*	5.40±1.68^{aA}	6.81±1.29^{aA}	6.10±0.75^{aA}
	Cube	8.97±2.27^{aA}	5.74±1.01^{aAB}	8.15±0.63^{aA}
360	*Cylinder*	3.07±0.97^{bA}	5.94±0.48^{abAB}	5.43±0.32^{aAB}
	Cube	7.22±0.90^{aA}	4.30±0.10^{bB}	5.20±0.46^{bB}
450	*Cylinder*	4.99±0.75^{aA}	4.56±0.22^{aB}	4.31±0.25^{aB}
	Cube	5.46±0.43^{aA}	7.98±1.18^{aA}	7.98±1.18^{aAB}
			Gumminess	
270	*Cylinder*	3.82±1.18^{aA}	4.90±0.85^{aA}	4.45±0.54^{aA}
	Cube	8.97±2.27^{aA}	5.74±1.01^{aAB}	8.35±0.50^{aA}
360	*Cylinder*	2.36±0.76^{bA}	4.27±0.40^{aA}	4.01±0.21^{aAB}
	Cube	7.22±0.90^{aA}	4.29±0.10^{bB}	5.20±0.46^{bB}
450	*Cylinder*	3.51±0.60^{aA}	3.60±0.30^{aA}	3.25±0.20^{aB}
	Cube	5.47±0.42^{aA}	7.99±1.18^{aA}	7.99±1.18^{aAB}
			Chewiness	
270	*Cylinder*	0.57±0.02^{aA}	0.60±0.01^{aA}	0.62±0.04^{aA}
	Cube	4.28±2.14^{aA}	1.42±0.22^{aAB}	2.18±0.08^{aA}
360	*Cylinder*	0.20±0.15^{bA}	0.53±0.07^{aA}	0.52±0.11^{aAB}
	Cube	1.80±0.38^{aAB}	0.68±0.20^{bB}	1.31±0.05^{abA}
450	*Cylinder*	0.46±0.08^{aA}	0.40±0.00^{aA}	0.36±0.35^{aB}
	Cube	0.70±0.05^{aB}	1.93±0.55^{aA}	1.93±0.55^{aA}
			Cohesiveness	
270	*Cylinder*	0.70±0.00^{aB}	0.71±0.01^{aA}	0.72±0.03^{aA}
	Cube	1.00±0.00^{aA}	1.00±0.00^{aA}	0.99±0.08^{aA}
360	*Cylinder*	0.76±0.02^{aA}	0.71±0.01^{bA}	0.73±0.00^{abA}
	Cube	1.00±0.00^{aA}	1.00±0.00^{aA}	1.00±0.00^{aA}
450	*Cylinder*	0.70±0.01^{bB}	0.73±0.02^{abA}	0.75±0.01^{aA}
	Cube	1.00±0.00^{aA}	1.00±0.00^{aA}	1.00±0.00^{aA}
			Springiness	
270	*Cylinder*	0.14±0.01^{aA}	0.11±0.01^{aA}	0.14±0.01^{aA}
	Cube	0.22±0.14^{aA}	0.24±0.02^{bA}	0.25±0.00^{abA}
360	*Cylinder*	0.07±0.04^{aA}	0.12±0.01^{aA}	0.12±0.02^{aA}
	Cube	0.24±0.02^{aAB}	0.26±0.04^{bB}	0.25±0.03^{aA}
450	*Cylinder*	0.13±0.01^{aA}	0.10±0.01^{aA}	0.10±0.01^{aA}
	Cube	0.48±0.10^{aB}	0.35±0.01^{aA}	0.35±0.01^{aA}
			Resilience	
270	*Cylinder*	0.39±0.01^{aA}	0.40±0.01^{aA}	0.38±0.02^{aB}
	Cube	0.16±0.04^{bB}	0.33±0.00^{aB}	0.34±0.01^{aA}
360	*Cylinder*	0.45±0.05^{aA}	0.40±0.00^{aA}	0.42±0.00^{aA}
	Cube	0.34±0.01^{aA}	0.33±0.50^{aA}	0.35±0.14^{aA}
450	*Cylinder*	0.39±0.01^{bA}	0.40±0.04^{abA}	0.46±0.01^{aA}
	Cube	0.35±0.02^{aA}	0.35±0.01^{aAB}	0.35±0.01^{aA}
F- value		Power	Time	Power × Time
Hardness	*Cylinder*	7.44*	4.83*	3.24*
	Cube	6.42**	2.52NS	5.46**
Gumminess	*Cylinder*	5.78*	5.87*	2.13NS
	Cube	6.85**	2.69NS	5.56**
Chewiness	*Cylinder*	6.32**	1.82NS	2.70NS

	Cube	7.26**	2.90NS	5.52**
Cohesiveness	*Cylinder*	3.70*	1.19NS	4.89**
	Cube	1.65NS	1.45NS	1.08NS
Springiness	*Cylinder*	3.69*	0.71NS	3.96*
	Cube	9.56***	2.35NS	6.89*
Resilience	*Cylinder*	4.08*	1.23NS	3.89*
	Cube	32.31***	18.08***	14.06***

Note: *Data presented as Mean ± SD values (n=3).* ^{abc}*Means within column (power) and* ^{ABC}*Means within a row (time) the same superscript is not significantly different, NS non-significant, *p<0.05, **p<0.01, ***p<0.001; n=3*

Table 4 One way ANOVA for sensory evaluation of Microwave in-package treated *Paneer* at different Power and Time

Microwave Power (W) ↓	Exposure Time (s) → Geometry	10	20	30
		Sensory attributes		
		Flavour		
270	*Cylinder*	48.33 ± 0.57^{abA}	49.66 ± 0.57^{aA}	47.66 ± 0.57^{bA}
	Cube	46.67 ± 2.30^{aA}	47.67 ± 0.57^{aA}	47.67 ± 0.57^{aA}
360	*Cylinder*	40.33 ± 0.57^{aB}	39.33 ± 0.57^{aC}	40.33 ± 0.57^{aC}
	Cube	47.33 ± 0.57^{aA}	47.33 ± 0.57^{aA}	46.67 ± 1.58^{aA}
450	*Cylinder*	40.33 ± 0.57^{cB}	44.66 ± 0.57^{aB}	42.33 ± 0.57^{bB}
	Cube	47.00 ± 0.00^{aA}	46.67 ± 0.57^{aA}	46.33 ± 0.57^{aA}
		Body & Texture		
270	*Cylinder*	30.00 ± 0.00^{bA}	32.00 ± 0.00^{aA}	31.66 ± 0.57^{aA}
	Cube	33.67 ± 0.57^{aA}	34.67 ± 0.57^{aA}	34.67 ± 0.57^{aA}
360	*Cylinder*	29.33 ± 0.57^{aA}	29.66 ± 0.57^{aB}	25.66 ± 0.57^{bB}
	Cube	34.33 ± 0.57^{aA}	34.33 ± 0.57^{aA}	35.00 ± 0.57^{aA}
450	*Cylinder*	26.33 ± 0.57^{aB}	27.66 ± 0.57^{aC}	27.00 ± 1.00^{aB}
	Cube	34.33 ± 0.57^{aA}	34.00 ± 0.00^{aA}	34.33 ± 0.57^{aA}
		Colour & Appearance		
270	*Cylinder*	9.33 ± 0.57^{aA}	9.33 ± 0.57^{aA}	09.66 ± 0.57^{aA}
	Cube	09.67 ± 0.57^{aA}	10.00 ± 0.00^{aA}	09.67 ± 0.57^{aA}
360	*Cylinder*	8.33 ± 0.57^{aAB}	7.66 ± 0.57^{abB}	06.00 ± 1.00^{bB}
	Cube	10.00 ± 0.00^{aA}	10.00 ± 0.00^{aA}	10.00 ± 0.00^{aA}
450	*Cylinder*	7.00 ± 1.00^{aB}	7.33 ± 0.57^{aB}	07.33 ± 0.57^{aB}
	Cube	09.67 ± 0.57^{aA}	10.00 ± 0.00^{aA}	09.67 ± 0.57^{aA}
		Overall acceptability		
270	*Cylinder*	92.66 ± 0.57^{bA}	96.99 ± 0.00^{aA}	93.98 ± 1.00^{bA}
	Cube	94.68 ± 2.30^{aA}	97.34 ± 1.14^{aA}	97.01 ± 1.00^{aA}
360	*Cylinder*	83.99 ± 1.00^{aB}	81.65 ± 1.71^{aC}	76.99 ± 2.00^{bC}
	Cube	96.33 ± 1.15^{aA}	96.33 ± 0.57^{aA}	96.34 ± 2.72^{aA}
450	*Cylinder*	78.66 ± 1.14^{aC}	84.65 ± 0.57^{aB}	81.66 ± 2.14^{bB}
	Cube	96.00 ± 1.00^{aA}	95.34 ± 0.57^{aA}	95.00 ± 2.28^{aA}
	F- value	Power	Time	Power × Time
Flavour	*Cylinder*	524.33***	17.33***	18.66***
	Cube	0.96^{NS}	0.24^{NS}	0.70^{NS}
Body & Texture	*Cylinder*	127.44***	20.11***	19.27***
	Cube	1.00^{NS}	2.71^{NS}	1.64*
Colour & Appearance	*Cylinder*	29.30***	1.61^{NS}	3.92^{NS}
	Cube	1.00^{NS}	1.00^{NS}	0.25^{NS}
Overall acceptability	*Cylinder*	19.41***	1.58^{NS}	2.64*
	Cube	11.37***	1.80^{NS}	1.60^{NS}

Note: *Data presented as Mean ± SD values (n=3).* ^{abc}*Means within column (power) and* ^{ABC}*Means within a row (time) the same superscript is not significantly different, NS non-significant, *p<0.05, **p<0.01, ***p<0.001; n=3*

cube shaped *paneer* the hardness, gumminess and chewiness were significant ($p<0.01$), springiness and resilience were highly significant ($p<0.001$) and cohesiveness was non-significant with time and it was also observed that all properties were non-significant with time.

The hardness ranged from 3.07±0.97 (360 W, 10s) to 6.81±1.29 N (270 W, 20 s) for cylinder shaped *paneer* whereas in case of cube shaped *paneer,* it was ranged from 4.30±0.10 (360 W, 20 s) to 8.97±2.27 (270 W, 10 s). So in both shaped *paneer* it was observed that hardness (N) decreased significantly as microwave power increased with respect at time. This may be due to softening of product, coupled with fusion and vapour/ moisture exudation (Badola et al. 2017). The hardness was statistically par with each other for all microwave power and time in both shaped *paneer*. The average values of cylinder shaped *paneer*, hardness, gumminess, springiness and chewiness was decreased significantly with increasing microwave power and time whereas cohesiveness and resilience were increases shown in Table 3. In case of cube shaped *paneer* the textural property such as springiness and resilience were increase with increasing microwave power and time and it ranged from 0.22±0.14(270W, 10sec) to 0.48±0.10 (450 W 10 s), 0.16±0.04 (270W, 10sec) to 0.35±0.01(450W, 30sec) respectively. The cohesiveness was shows the constant value for all microwave power and time i.e. 1.00±0.00.

Sensory characteristics of in-package microwave treated *paneer*

Sensory characteristics of in-package microwave treated *paneer* are represented as values in Table 4. The flavour score was highly significant ($p<0.001$) with microwave power, time and their interaction for cylinder shaped *paneer* and for cube shape it was observed non-significant effect with power, time and their interaction, The flavour values ranged from 39.33±0.57 (360W, 20 s) to 49.66±0.57 (270W, 20 s) for cylinder shape *paneer* and for cube shape *paneer*, it ranged 46.33±0.57 (450W, 30s) to 47.67±0.57.

The Body and texture was also follow similar trends like flavour. The body and texture values was ranged from 25.66±0.57 (360W, 30s) to 32.00±0.00 (270W, 20s) for cylinder shaped *paneer* and for cube shaped *paneer*, it ranged from 33.67±0.57 (270W, 10s) to 35.00±0.57 (360W, 30 s).

The colour and appearance values of cylinder shaped *paneer* were highly significant with power and non-significant with time and their interaction and for cubed shaped *paneer* there was non-significant effect with power, time and their interaction. The colour and appearance values were ranged from 6.00±1.00 (360 W, 30 s) to 09.66±0.57 (270W,30 s) for cylinder shaped *paneer* and in case of cube shaped *paneer*, it ranged from 09.67±0.57 (450W, 10 s) to 10.00±0.00 (360W, 10s). The sensory score for overall acceptability presented a highly significant ($p<0.001$) for microwave power and non-significant for time in both shaped.

The overall acceptability values for cylinder shaped *paneer* ranged from 76.99±1.00 (360W, 30s) to 96.99±0.00(270W, 20s) and that of cube shaped *paneer* it was ranged from 94.68±2.30(270W, 10s) to 97.34±1.14 (270 W, 20 s).

From overall acceptability it was observed that as microwave power and time was increased the score was also increase in both shaped *paneer*.

Conclusions

The product *paneer* (cylinder and cube shaped) was packed in polypropylene subjected to the microwave treatment at different power (270, 360 and 450W) and exposed for different time (10, 20 and 30s). It was observed that all properties which seem to be important with the point of view of quality of *paneer* such as whiteness index, hardness, flavour and overall acceptability were in higher in cube shaped *paneer* than those in cylinder shaped. Thus it could be concluded that for in-package microwave treatment a cube shaped *paneer* can be use over cylindrical shape. The overall acceptability values for cylinder shaped *paneer* ranged from 76.99±1.00 (360W, 30s) to 96.99±0.00(270W, 20s) and that of cube shaped *paneer* it was ranged from 94.68±2.30 (270W, 10s) to 97.34±1.14 (270 W, 20s).

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RESEARCH ARTICLE

Development and characterisation of yoghurt incorporated with Dates (*Phoenix Dactylifera Linn*) extract

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Abstract: Nowadays, functional dairy products gain more acceptance in the market because of the additional health and nutritional benefits they provide to the consumer. Value addition of dairy products like yoghurt often augments the market value as well as functionality of the product. Dates (*Phoenix dactylifera Linn*) are natural source of phytochemicals like phenolic acids and flavonoids which are proved to have strong anti-oxidant activity. In this study, the effect of addition of dates extract into yoghurt milk was studied. Hot water extract of dates fruit was added to the yoghurt at different levels (30%, 32%, 34%, and 36%) and the optimum level of addition was selected on the basis of sensory scores obtained from 5 semi trained judges. Significant differences ($p<0.01$) were found in the sensory scores of different treatments and the overall highest score was obtained for yoghurt with 34% dates extract. The product selected was subjected for physico chemical analysis and the findings were: fat 2.85%, protein 3.8, total solids 15.88 and acidity 0.79% Lactic acid (LA). The total phenolic content of the product was measured in terms of milligrams of Gallic acid equivalents (GAEs) per 100 grams of sample (mg GAE/g of sample) and was found to be 59 mg GAE/100g. The phenolic contents in the product was proved to impart antioxidant activity in the yoghurt as the IC 50 value; the half maximal inhibitory value, was found to be 19.99mg/mL of the sample.

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Keywords: Anti-oxidant activity, Dates extract, Yoghurt, Total phenolic content

Introduction

Rising interest in healthy eating habits has led to a new array of food products on the market which, despite of providing nutritional benefits, adds to improvement in health and reducing the risk of certain diseases. Internationally accepted dairy products like yoghurt can act as a medium for the incorporation of functional components like poly-phenolic compounds (Chouchouli et al. 2013). Yogurt is obtained by the fermentation of milk by *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus*. It is a good source of essential micro and macro nutrients including bioactive peptides, with good anti-oxidant properties produced during fermentation (Granato et al. 2010)

Yoghurt is considered to be a product with high functionality owing to the presence of living microorganisms such as bifidobacteria, lactic acid bacteria (LAB), streptococci or their combinations. Fermented milk products comprising yoghurt are proven to be good sources of minerals like calcium and potassium as well as proteins (Dimidi et al. 2019). Moreover, yoghurt also plays a potential role in decreasing intestinal disorders and chronic diseases (Balakrishnan et al. 2016).

However, plain yogurt contains very less phenolic and anti-oxidant compounds and their impact on human health is of little significance. In order to improve the nutritive value of yoghurt, it is often added with antioxidants from natural sources which provides additional health benefits to it. Hence, a number of attempts to prepare yoghurts fortified with natural antioxidant-rich extracts have been done by the functional food industry (Chouchouli et al. 2013). The potential useful components of functional ingredients for this fortification are carotenoids, Vitamin C, flavonoids, Vitamin E and other polyphenols (John and Singla 2021). Plants synthesise natural functional compounds including polyphenols as secondary metabolites as a response to environmental stress. The phenolic components in foods offer many possible beneficial effects on human health and therefore are the subject of increasing scientific interest. Various studies

have shown that diets rich in plant polyphenols, when consumed for a long term contributed to the prevention against the development of cancers, osteoporosis, neurodegenerative diseases, diabetes, and cardiovascular diseases (Pandey and Rizvi 2009). Yoghurt added with natural extracts or concentrates of fruits like dates, grapes, passion fruit etc, are known to have good anti-oxidant activity and poly phenol contents (Amerinasab et al. 2015). Dates fruit (*Phoenix dactylifera L.*) is usually available in market as ready-to-use, semi-finished products or products like date juice, date syrup, date spread and date liquid sugar (DLS). The dried dates fruit contain a high percentage of carbohydrates (total sugars, 66.1 to 88.6g/100g), fat (0.1 - 1.4g/100g), protein (1.6–3g/100g), energy (258 –344kcal/100g), ash (1.3-1.9g/100g). Dates also contain good number of antioxidants, primarily carotenoids and phenolics. It also contains a considerable amount of dietary fiber (8 g/100 g), antioxidants (80400 µmol/100 g) and phenolics (3942 mg/100 g) (Al-Farsi and Lee 2008). The unsaturated fatty acids existent in dates are linoleic, oleic and linolenic acids. Dates also contain minerals like magnesium, copper, selenium and potassium and vitamins like Vitamins C and B-complex.

The objective of this study was to fortify a yogurt with natural extract of dates fruit in order to improve the functionality of the product as well as to improve the sensory attributes of the yoghurt. The product was prepared by adding the hot water extract of dates fruit before inoculation which was followed by incubation after which the sensorial and quality attributes of the formulated product was compared with plain yogurt.

Materials and Methods

Preparation of Dates extract

Good quality dates were procured from the local market and was cleaned properly. Dates, after removing the seeds, were crushed using a mortar and pestle. The crushed dates was infused with 2 times of hot water and then stirred for 2 hours. The mix was allowed to stand for overnight in cold place to obtain the complete extract. The dates-water mix was then boiled until the tissue softened and the extract was obtained by squeezing the mix using sterilised muslin cloth. The hot water extract of dates was bottled and used.

Preparation of Dates incorporated yoghurt

Cow milk was procured from the Kerala Veterinary and Animal Sciences University Dairy Plant, Mannuthy, Thrissur. Yoghurt culture was procured from Dairy Microbiology Laboratory, Kerala Veterinary and Animal Sciences University. The total solids content of the milk was standardised to about 13% by the addition of skim milk (3% fat and 10% SNF). Preliminary trials were conducted to select the maximum and minimum levels of incorporation of the dates extract into the milk. Based on these preliminary trials, four levels (30%, 32%,34% and 36% of the milk) were considered for further sensory analysis in 4 replications. The milk used to make yoghurt was heated to 90p C for 15 minutes followed by cooling to 42°C. Dates extract was added to milk at different levels followed by inoculation with yoghurt culture, 1:1 mixture of *S. Thermophiles and L. bulgaricus* and then the cultured mix was incubated at 42°C for 4 hours.

Sensory evaluation

Sensory evaluation of the samples was carried out by a panel of 5 semi trained judges. The parameters of study were the color and appearance, body and texture, flavour, acidity and overall acceptability. The sensory evaluation was carried out based on 9-point Hedonic scale in which a score of 1 represented 'dislike extremely' and score of 9 represented 'like extremely'. The samples for analysis were presented before the judges after suitable marking. The judges were provided with a room with good lighting and appropriate facilities. The selection of optimum product from all the treatments was done based on the sensory scores obtained.

Texture Profile analysis

Texture analyser (Shimadsu, Model EZ-X series, Japan) fitted with a load cell of 200N and a cylindrical probe (25.4 mm in diameter) connected to the software TRAPEZIUM X was used to determine the texture profile of yoghurt. Samples were kept at 25 °C prior to TPA analysis. TPA was performed by using a probe to compress twice and achieve a penetration of 10 mm at a velocity of 1 mm/s. Hardness, adhesiveness, cohesiveness and gumminess were determined from TPA by using software. All measurements were carried out in triplicate for each sample.

Proximate composition analysis

The milk used for yoghurt preparation as well as the dates incorporated yoghurt were subjected for proximate analysis. The total solids and protein content of the samples were determined as per IS: 1479 (part-II), 1961.The fat content was determined by using standard Gerber method as per IS: 1224 (part-I), 1977 and the acidity was estimated according to IS: 1479, (part–I), 1960.

Total phenol content and anti-oxidant activity

Total polyphenols in the dates extract and the dates yoghurt were determined by employing the Foline Ciocalteu assay, as described by Arnous et al. (2002). Gallic acid was taken as the reference standard, and results were expressed as mg gallic acid equivalents (GAE) per g fresh weight (mg GAE/g of sample).

The DPPH assay was conducted for the determination of anti-oxidant activity in the developed product. The assay measures the reducing ability of the anti-oxidants present in the product towards the DPPH radical. A decrease in DPPH radical absorption

is indicated by a change in hue from purple to yellow. This shows that antioxidants in a mixed solution interact with free radicals. The anti-oxidant activity of dates yoghurt was determined using the procedure given by Brand et al. (1995). A 100ul aliquot of the sample was vortexed with 2.9ml of 60Um DPPH (2, 2- diphenyl-1-picrylhydrazyl) solution in methanol. After 30 minutes in the dark, the absorbance of the mixture was measured at 517 nm. As a control, methanol was employed. The IC (Inhibition Concentration) 50 values for antioxidant activity were given in milligrammes per litre. The concentration of anti-oxidant in the test solution was related to the decrease in DPPH absorbance compared to blank determined spectrophotometrically at 516nm. The percentage of inhibition of DPPH oxidation was calculated according to the formula:

$$\text{DPPH Scavenging effect (\%)} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Color characteristics

MiniScan EZ spectrophotometer was used for the measure of color parameters of the yoghurt samples. Both the control and optimized samples of yoghurt were tempered to room temperature. The samples were filled in petriplates for taking measurement. The color characteristic parameters such as 'L*', 'a*' and 'b*' values were noted; The colour parameter 'L* ' is a measure of lightness or luminance, which ranges from 0 to 100 [L* = 0 (black) and L* = 100 (white)] and 'a* ' and 'b* ' are the two chromatic components, which range from -120 to -120 [a* = -120 (green) and a * = 120 (red)] and [b* = -120 (blue) and b* = 120 (yellow)] respectively.

Statistical analysis

The results were analysed using SPSS v.16.0 for windows software. The sensory scores obtained for different treatments while optimising the product, were subjected to Kruskal – Wallis test followed by Mann-Whitney u-test. The data obtained during compositional analysis of the optimised dates yoghurt and the control was compared using independent t test.

Results and Discussion

Sensory evaluation

Preliminary trials were conducted by incorporating dates extract at different concentrations in milk intended for the preparation of yoghurt. The minimum and maximum level of incorporation of dates extract in milk was selected as 30% and 36% respectively. The sensory scores obtained for different treatments were statistically analysed and given in Table 1 and the graphical comparison of the sensory scores are given in figure 1. The treatments T1 depicts 30% dates extract, T2 depicts 32% extract, T3 represents 34% extract and T4 expresses 36% extract in milk.

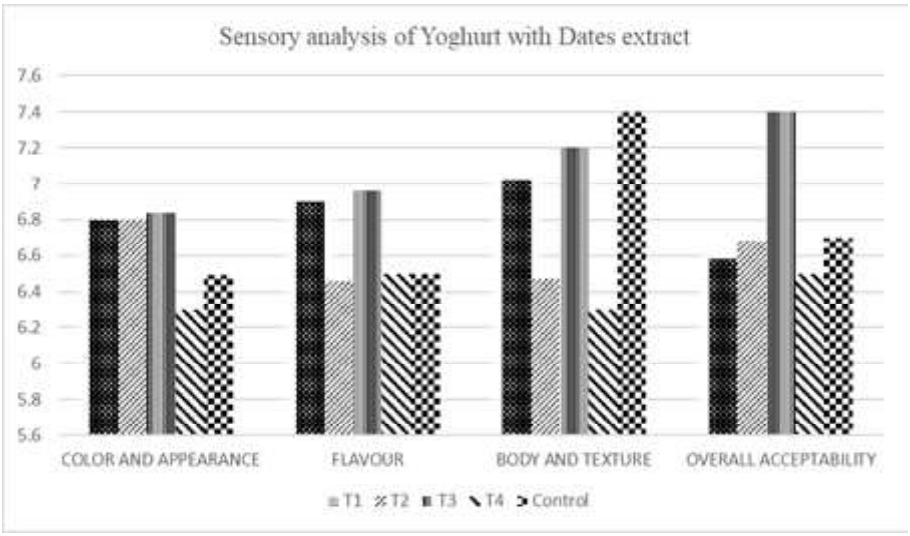

Fig. 1 Sensory analysis of dates incorporated yoghurt and the control sample

T1 - 30% dates extract added yoghurt, T2- 32% extract added yoghurt, T3 -34% extract added yoghurt and T4-36% extract added yoghurt

Table 1 Sensory analysis data of treatment samples and control yoghurt

Parameters	Sensory scores					
	T1	T2	T3	T4	Control	Chi square value
Color and appearance	6.8±0.131^{a}	6.8±0.077^{a}	6.84±0.092^{a}	6.30±0.122^{b}	6.49±0.032^{b}	20**
Flavour	6.9±0.015^{a}	6.46±0.128^{b}	6.96±0.081^{b}	6.5±0.058^{b}	6.50±0.158^{b}	16.9**
Body and Texture	7.02±0.159^{a}	6.47±0.012^{b}	7.02±0.122^{a}	6.3±0.111^{b}	7.40±0.100^{a}	12.2**
Overall Acceptability	6.58±0.073^{a}	6.68±0.096^{a}	7.4±0.100^{b}	6.5±0.158^{a}	6.70±0.122^{a}	22.6**

**- Significant at one per cent level ($p<0.01$), *- Significant at five per cent level ($p<0.05$), a-b Figures in a row bearing different superscript differ significantly, T1 - 30% dates extract added yoghurt, T2- 32% extract added yoghurt, T3 -34% extract added yoghurt and T4-36% extract added yoghurt. Figures are the mean ± standard error of sensory scores by five semi trained judges in four replications

The chi square values obtained in the study were found to be significant ($p<0.01$) indicating significant differences between the samples with respect to all the attributes. Highest score for flavour was secured by the yoghurt with 34% (T3) dates extract as compared with other treatments and control. However, the control sample secured the highest score for body and texture as the treated samples had less firm body compared to it. However, yoghurt with 34% dates extract had comparable scores to that of the control yoghurt. The scores obtained for color and appearance was moderately same for all the treated samples except for yoghurt with 36% dates extract. From the overall sensory scores obtained, yoghurt with 34% dates extract (T3) was selected as the optimum product and was subjected for further physicochemical analysis.

Amerinasab et al. (2015) studied the sensory characteristics of yoghurt added with date liquid sugar (DLS), obtained from refined dates syrup and concluded that increasing concentration of DLS in yoghurt beyond 6 per cent level resulted in decreasing firmness of the product. This decrease in firmness was found to be associated with the higher rate of syneresis and lower viscosity, caused by the addition of higher amounts of DLS.

Texture Profile analysis

It is proved that the structure of food has significant impact on various aspects, including functionality, texture and appearance. The composition and micro structure of the protein network determine the textural and rheological characteristics of fermented dairy products. (Delikanli and Ozcan, 2017, Kose et al. 2018). The data obtained for textural analysis (Table 2) showed that the textural parameters of all the treatments differ significantly with that of the control yoghurt. A notable difference was observed in the hardness of the treatment samples as compared to the control which was found to be decreasing with increase in the level of dates extract. This descending manner of hardness can be associated with the reduction in total soilds. Various studies also reported that, a decrease in hardness may be directly related to the fat content of yoghurt. (Berber et al. 2015, Lucey, 2014). Considering the dilution occurred on addition of dates extract, control yoghurt had higher percentage of fat which may have contributed to the much higher hardness than the treated samples. The hardness of yoghurt was found to fall within the range of 0.22N to 1.54N for traditional yoghurt and 0.94N to 224.62N for industrial yoghurt samples (Kose et al. 2018). Cohesiveness in semi-solid materials refers to the extent to which product sustains a second deformation in compared to how it reacted to the initial deformation (Ashu and Pradyuman, 2013). Cohesiveness of the yogurt showed a significant decrease with increasing levels of dates extract. This decrease may be caused by the dilution occurring due to the incorporation of dates extract. Reduced cohesion may be a result of the system's decreased viscosity (Mudgil et al. 2017).

Adhesiveness is defined as the negative force area for the first bite and depicts the effort required to overcome the attraction forces between the surface of a food and the surface of other materials with which the food comes into contact (Kasapis, 2009). It is the force required to remove the food material that adheres to the mouth while eating (Ganesh, 2006). The control yoghurt and treatment 1 (T1) had minimum adhesiveness as compared to the other treatment samples. The more negative the value of adhesiveness, the more "sticky" is the sample (Armero and Collar, 1997). A significant difference was found in the gumminess values of control yoghurt and the treatment samples. The force required to break down a semisolid food into a state suitable for ingesting is known as gumminess, which is measured by multiplying hardness and cohesiveness. (Yang and Li, 2010, Najgebauer-Lejko et al. 2015). As in the case of hardness, the treatment samples had a very lesser value of gumminess when compared with the control yoghurt. A similar decreasing trend in gumminess value was observed by Mendes et al. (2019) in yoghurt incorporated with Yakon syrup and cashew apple extract. As the level of incorporation increased, the hardness and gumminess value were decreased.

Proximate composition analysis

The cow milk used for the preparation of yoghurt was standardised to 13% total solids. The acidity was found to be 0.14 per cent lactic acid. The chemical quality of the optimised sample with 34% dates extract and control yoghurt is given in Table 2. The total soluble solids (TSS) content of the dates extract was found as 58p Brix using a hand refractometer. The inclusion of this dates extract may be responsible for the significant difference ($p<0.01$) in total solids between the dates yoghurt and the control. A significant difference ($p<0.01$) was observed in the fat content owing to the dilution occurring in the sample with addition of dates extract. Jung et al. (2016) observed similar

Table 2 Textural quality of treated samples of dates incorporated yoghurt

	Control	T1	T2	T3	T4	F value
Hardness (N)	0.98±0.009^a	0.284±0.142^b	0.271±0.126^b	0.260±0.120^c	0.215±0.107^d	2428.6**
Cohesiveness	0.543±0.271^a	0.527 0.263^b	0.526 0.263^b	0.48 0.015^c	0.477±0.238^c	43.8**
Gumminess	0.532±0.002^a	0.150±0.068^b	0.142±0.066^b	0.125±0.016^c	0.103±0.059^c	95.3**
Adhesiveness (J)	-0.0003±0	-0.0003±0	-0.0001±0	-0.0001±0	-0.0001±0	

**- Significant at one per cent level ($p<0.01$), *- Significant at five per cent level ($p<0.05$), a-b Figures in a row bearing different superscript differ significantly, T1 - 30% dates extract added yoghurt, T2- 32% extract added yoghurt, T3 -34% extract added yoghurt and T4-36% extract added yoghurt. Figures are the mean ± standard error of sensory scores by five semi trained judges in four replications

Table 3 Chemical quality of yoghurt incorporated with 34% dates extract and control

	Chemical composition		
	Dates yoghurt	Control	t value
Fat (%)	2.85±0.022	3.08±0.0.083	5.2**
Protein (%)	3.8±0.141	3.91±0.008	1.7^{ns}
Total solids (%)	15.88±0.083	15.48±0.083	7.5**
Acidity (% LA)	0.79±0.005	0.63±0.015	9.95**

Figures are mean ± standard error of three replications, *-Significant at one per cent level ($p<0.01$), ns – non significant

Table 4: Color characteristics of dates incorporated yoghurt and the control sample

Parameter	Dates Yoghurt (T3)	Control sample	t value
L*	82.04±0.160	89.02±0.045	-41.9**
a*	0.62±0.003	2.41±0.003	-166.7**
b*	17.8±0.063	14.66±0.031	44.4**

Figures are mean ± standard error of three replications, *-Significant at one per cent level ($p<0.01$), ns – non significant

decrease in the fat content and increase in total solids content in a study conducted on yoghurt added with red ginseng extract. Whereas the protein content in both the control and dates yoghurt tend to remain same with no significant difference ($p<0.01$). Similar insignificant decrease in protein content was observed by Gad et al. (2010) in a study conducted in yoghurt added with date palm syrup and skim milk.

Similarly, acidity of the control yoghurt was found to be 0.63% lactic acid which differed significantly ($p<0.01$) with the acidity of dates yoghurt, 0.79% lactic acid. This could be because the addition of dates extract, which contains simple carbohydrates like glucose and fructose, activated the metabolic activity of the added culture (Amerinasab et al. 2015)

Total phenol content and anti-oxidant activity

Dates contain considerable amount of phenolic compounds including flavonoid glycosides, anthocyanins, flavanols, and proanthocyanidins etc which are proved to exhibit act very active antioxidant properties (Ghnimi et al. 2017, Vayalil 2012, Mansouri et al. 2005). According to various studies (Ismail, 2021; Matloob and Balakit, 2016; Vayalil 2012), the total phenolic content of different varieties of dates ranged from 19.88 to 475 mg GAE/100 g, The dates utilised in the current study had a total phenolic content of 198 mg GAE/100 g which was in agreement to the previous findings. Also, the total phenolic content of the optimised dates yoghurt was found to be 59.23mg GAE/100g which indicates that much of the phenolic content in the dates has been passed on to the yoghurt incorporated with it. Similarly, the yoghurt added with dates liquid sugar had phenolic content and was shown to have 0.03µg GAE/mg of yoghurt (Amerinasab et al. 2015).

In terms of antioxidant activity, a strong link was discovered between phenolic content and antiradical efficiency by Mansouri et al. (2005). The fruit extract of dates exhibits a high level of free radical scavenging action and dates extract has proven to be an effective scavenger of reactive oxygen species such as hydroxyl (OH•) and superoxide (O•–) radicals, as well as a potent inhibitor of in vitro macromolecular damages like protein oxidation and lipid peroxidation (Vayalil 2002). The total anti-oxidant capacity of the sample was tested using the DPPH radical (2, 2-diphenyl-1-picryl hydrazyl radical) as per the procedure given by Brand et al. (1995).

The anti-oxidant ability of the sample was measured in terms of IC50 value, i.e, the half maximum inhibitory concentration, which is a measurement of the substance's ability to inhibit a biological or metabolic function. This value specifies how much of a particular component or inhibitor is required to inhibit a biological substance existing in the medium by half. The smaller IC50 value, the higher the radical scavenging rate, hence higher will be the antioxidant capacity. (He et al. 2010). The IC 50 value obtained for the dates yoghurt sample was found to be 19.99mg/100ml. Plain yoghurt tend to have a very lower antioxidant activity. A study conducted by Ye et al. (2013) proved that the antioxidant activity of the plain cow milk yoghurt prepared with 1:1 mixture of *S. Thermophiles and L. bulgaricus* culture exhibited slightly higher IC 50 values (4800mg/100ml) when compared to the yoghurt added with hickory-black soybean yoghurt (4285mg/100ml). On comparison with the IC 50 value obtained for plain yoghurt in the former study, it can be inferred that the addition of dates imparted very high anti-oxidant ability to the dates yoghurt. This high antioxidant activity of the dates yoghurt can be attributed to the phenolic components in dates extract like phenolic acids including ferulic, p coumaric, isoferulic, vanillic acids and flavonoids like quercetin, luteolin, apigenin etc. (Eid et al. 2014), (Pandey and Rizvi 2009)

Color characteristics

The color characteristics of control and dates yoghut are given in Table 4. It can be inferred that the addition of dates extract influences the color parameters of yoghurt ($p<0.01$). The lightness

value (L*) of control and dates yoghurt was found to be 89.02 and 82.04 respectively and hence it can be inferred that the addition of dates extract has reduced the lightness value of yoghurt. The mean values of chromatic components a* and b* of control sample was found to be 0.62 and 17.8 respectively while that of the optimised sample was found to be 2.43 and 14.66 respectively. Here, the a* value is higher for the treated sample which indicates more redness for the sample than the control. The b* value is lower for the treated sample as compared with the control which indicates more yellowness for the control than the sample. Dates consists of color components like melanoidines, and iron polyphenolic complexes which may contribute to the yellow and red colour of dates extract (Fathi et al. 2018). Similar observations were made by Sert et al. (2011) in a study conducted in yoghurt incorporated sunflower and honey into set-type yoghurts.

Conclusions

Dates fruit is renowned for its therapeutic values as it contains many classes of bioactive components such as carotenoids, polyphenols especially phenolic acids isoflavons, lignans, and flavonoids, tannins, and sterols etc. Value addition of yoghurt and other dairy products is gaining much importance in the present scenario. Incorporation of dates extract to yoghurt not only improves the flavour but also enhances the nutritive quality of the product. However, the textural analysis of all the treatments along with the control yoghurt suggested that the textural properties, especially firmness or hardness was deleteriously affected with the increase in levels of dates extract. Meanwhile as the sensory scores obtained was higher for the treatment with 34% dates extract, it was selected as the optimum product for further physicochemical analysis. The total phenolic content in dates yoghurt suggested that a considerable amount of phenolics in dates extract was transferred into the product. It was also proved that the dates yoghurt showed anti-oxidant activity, which can be attributed to the phenolic compounds present in dates like ferulic acid, vanillic acid, coumaric acid etc. Considering the textural quality, yoghurt added with dates extract would find more appropriate use as drinking or stirred type than set type yoghurt. Further studies may be done to scale up the study on this product for its commercialisation.

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RESEARCH ARTICLE

Development and evaluation of sensorial and antioxidant properties of functional black rice *kheer*

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Abstract: The present investigation involved preparation of *kheer* by replacing white rice with black rice @ 40g (K1), 30g (K2) and 20g (K3). Coconut sugar was added @15 g in all the combinations. Standardized pasteurized milk (4.5% fat and 8.5% SNF) was used with black rice grains. Cleaned rice was soaked in water (rice to water ratio as 1:2) and cooked at 93°C till the water was completely absorbed. *Kheer* prepared using 20g of rice (K3) was selected as optimized product on the basis of sensory evaluation. The developed black rice *kheer* was acceptable upto 12 days of storage at refrigerated temperature. Optimized *kheer* contained 58.45±0.04% moisture, 5.11±0.26% fat, 72.57±0.51% total carbohydrate and total phenolic content 54.46±0.56 (mg of gallic acid equivalents/g dry weight). During storage at refrigerated temperature (4±2°C), the pH decreased significantly while acidity increased. The SPC increased from 1.32±0.3 to 6.89 ± 0.23 (log CFU/g) at the end of 12 days storage in optimized black rice *kheer*. This study can be utilized to find out the suitability of black rice which is known to have many functional properties, to the non-consumers of black rice and to add variety to the diets of black rice consumers.

Keywords: *Kheer*, Black rice, Functional, Total phenolic content, Traditional dairy product

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Introduction

Now a day's black rice (*Oryza sativa* L.) is widely used among the health-conscious consumers as a 'functional food' due to its nutritional profile. Black rice is good source of protein, iron, antioxidants and vitamin E (Kumar and Murali, 2020). Black rice is a potential functional food ingredient owing to its useful composition such as very low fat and sugar, gluten free and high protein (Sushmitha and Reddy, 2020). Priya et al. (2019) compared the nutritional profile of black rice with red, brown and white rice and found that black rice has the highest amount of fat (2.0g/100g), protein (8.50g/100g), tocopherols (12.54 mg/100g), thiamine (0.46 mg/100g), riboflavin (0.403 mg/100g) and zinc (3.16 mg/100g). Chanu et al. (2016) reported that black rice is free from gluten and has higher protein per cent (11.40), fat per cent (3.20), crude fibre per cent (1.64), whereas zinc (6.2mg/100g) and manganese (2.56%) content is higher in Chakhaoamubi and Poireitonchakhao variety of black rice, respectively.

Black rice has been regarded as an excellent source of anthocyanins, which can reduce the risk of cancer, obesity and its compounds has anti- ageing, antiviral, anti-inflammatory effects (Yamuangmorn and Prom-u-Thai, 2021). Research has shown that black rice has many health benefits. Anthocyannins are the characteristic compound which is responsible for its popularity throughout the world. More than 90% of the total anthocyanins are constituted by cyanidin-3-glucoside in black rice, followed by peonidin-3-glucoside (Zhang et al. 2010). Balasubramaniam et al. (2019) reported the health benefits of black rice such as its antioxidant activity, anti-proliferative, anticancer, anti-diabetes, anti-atherosclerosis and cholesterol lowering activity. Sterols and triterpenoids found in black rice have potential anticancer properties (Dias et al. 2017).

Kheer is a very popular traditional dairy product consumed all over India. It is considered as nutritious dairy dessert. But its production is limited to household level and unorganized sector only. Coconut sugar has been reported to have glycemic index of about 35 (Kusumawaty et al. 2012). It is a good source of minerals like zinc, iron, calcium, phosphorous, potassium and magnesium. Hebbar et al. (2015) also reported that coconut sugar is good source of vitamins, such as vitamin C, B-complex, antioxidants,

polyphenols and dietary fibres. Recently, coconut sugar become very popular among health conscious consumers.

In this study, black rice in different proportions was added to replace the white rice for preparation of *kheer*. The major objective of this study was to popularize the consumption of black rice in the areas where it is not consumed. There are some studies on the enrichment of *kheer* with millets, quinoa, pumpkin etc., but there are no studies on the *kheer* preparation using black rice. Hence, the present study was undertaken to prepare and evaluate *kheer* by incorporating different proportions of black rice in traditional white rice-based *kheer*.

Materials and Methods

Black rice (Chahao) was procured from market of Shalimar Bagh, New Delhi. Organic coconut sugar of Tropicoco Kokos Natural was purchased through online shopping site (Flipkart). Standardized & pasteurized milk (with fat: 4.5% and SNF:8.5%) was procured from local market of Varanasi, Uttar Pradesh. Chemicals used in the analysis were procured from different sources Hi -Media Laboratories Pvt. Ltd., Mumbai, India; Sigma Chemicals Co. St. Louise, M.O., USA; Fisher scientific, Mumbai, India and Merck Specialists Pvt. Ltd., Mumbai, India.

Preparation of rice powder

Rice was cleaned and milled to a fine powder using mixer grinder and then the powder was transferred to low density polyethylene (LDPE) packages of size 12 cm x 9 cm, sealed and vacuum packed for further study.

Nutritional composition and antioxidant activity of black rice

By following the method of AOAC (2000), moisture and fat content was calculated by taking 5.0 g of sample. The ash content of finely ground sample of black rice was estimated by following the method of AOAC 2000. Iron and zinc were analyzed using Atomic Absorption Spectroscopy (AAS) (Thermo Fisher Scientist-IN) (Mowuta, and Mayangsari, 2022). Sample of rice flour was extracted by method of Sutharut and Sudarat (2012). Total phenolic content (TPC) was determined using the modified method of Folin-Ciocalteau method (Lavelli et al. 2016). The DPPH radical scavenging assay was performed according to the method of (Locatelli et al. 2009).

Preparation of black rice *kheer*

Firstly, rice was soaked (1part of rice:2 part of water) for half an hour and cooked in a cooking pan with occasionally stirring with a wooden spoon till the water was completely absorbed/ evaporated. The flowchart for preparation of black rice *kheer* is given in (Figure 1). The proportions of ingredients produced by the levels of black rice addition were considered as treatments given below (Figure 2). The proportions used were decided based on the preliminary trials. Notations for control and different treatments are shown below:

K_0 = White rice (12.5g) + Sugar (25g) + Toned Milk (500ml) as control

K_1 = Black Rice (40g) + Coconut Sugar (15g) + Toned Milk (500ml) + Cardamom pod (1)

K_2 = Black Rice (30g) + Coconut Sugar (15g) + Toned Milk (500ml) + Cardamom pod (1)

K_3 = Black Rice (20g) + Coconut Sugar (15g) + Toned Milk (500ml) + Cardamom pod (1)

Sensory evaluation

The samples were evaluated for their sensory attributes i.e. flavour, colour, texture/body, and overall acceptability by semi - trained panel of 20 judges using 9 point hedonic scale. A score of 5.5 and above was considered acceptable. The scores rated by the panel of judges were then statistically analyzed. The samples were code numbered to avoid identification and bias. Based on sensory score K_3 sample was found most acceptable and therefore it has been selected for further study.

Chemical composition analysis of black rice *kheer*

By following the method of AOAC (2000), moisture content was calculated by taking 5g of sample. After the determination of moisture, the left residue was taken for calculation the total solid content.

Determination of total phenolic content

The TPC of extracts was determined using the Folin-Ciocalteu's phenol reagent. 0.5 ml of diluted extract was added to 2.5ml of 0.2N Folin-Ciocalteau reagent and placed for 5 minutes. 2ml of 75g/L of Na_2CO_3 was then added. The above solution was then kept for incubation at room temperature for 2 hours. Absorbance was measured at 760nm using a 1cm cuvette UV-1800 spectrometer (Shimadzu, Japan). Gallic acid (0-800mg/L) was used to produce a standard calibration curve. The total phenolic content was expressed in mg of Gallic acid equivalent (GAE)/100ml of extract after applying the dilution factor (Stankovic, 2011). The free radical scavenging activity was determined by using DPPH assay with modified method of (Brand-Williams et al. 1995).

Statistical Analysis

The data obtained during the course of investigation were subjected to statistical analysis. One-way analysis of variance (ANOVA) was applied to analyze test of significance.

Fig.1 Diagrammatic representation of different combinations of black rice *kheer*

Shelf- life of black rice *kheer*

Based on the sensory characteristics of the different combinations K_3 was selected for shelf- life study with control sample of white rice *kheer. Kheer* was packed in polystyrene cups of 100 g capacity and stored at 4±2°C in refrigerator. The pH, acidity and microbial parameters of black rice *kheer* were determined at an interval of 2 days for a period of 12 days.

Results and Discussion

The ash content was high in black rice (2.80%). The amount of ash content indicates the levels of minerals present in the food sample. Similar results were reported by (Colasanto et al. 2021). The authors reported that the moisture content of black rice was 11.70%, total dietary fiber, proteins, and ashes were 10.8%, 10.5% and 1.95% on dry weight (d.w.), respectively. The study conducted by Thomas et al. (2013) reported that fat content of black rice was 0.70% but in the present study the fat content was 1.90%. The difference in fat content may be due to the cultivation and regional difference (Kang et al. 2011). Reported mineral content of black rice was zinc (6.20mg/100g) and iron (3.50 mg/100g).

Plant phenolic are important constituents that contribute to functional quality, colour and flavour and have significant roles in both as singlet oxygen quencher and free radical scavengers, helping to minimize molecular damage (Tanvir et al. 2017). Phenolic contents were expressed as mg gallic acid equivalents per gram of black rice extract. Using the standard curve, total phenolic content of the extract was determined. The total phenolic content of the black and white rice extracts was found to be 75.59±7.28 and 11.94±0.97 mg GAE/g of black rice extract & control extract, respectively. The results indicate that the black rice extract contains higher total phenols as compare to the white rice extracts. The phenolic constituents of rice are mainly distributed in rice pericarp (Paiva et al. 2014). This may be the reason for higher phenolic contents in black rice than the white rice. The main form of phenolic compounds present in rice is bound form (Saikia et al. 2012). The free radical scavenging activities of the extracts were determined by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. DPPH in oxidized form gives a deep violet color in ethanol. An antioxidant compound donates the electron to DPPH thus causing its reduction and in reduced from its color changes from deep violet to yellow (Shirazi et al. 2014).

Fig. 2 Flow chart for preparation of functional black rice *Kheer*

Based on % DPPH assay, the antioxidant capacity of the extracts were found to be 56.23±3.56 & 11.67±1.18 for black rice and white rice extract, respectively. From Table 1, it can be concluded that antioxidant capacity of black rice is much more than that of white rice. These results were comparable with the observations given by Sampong et al. (2011) and Walter et al. (2013) i.e. DPPH value ranged from 59.02 to 75.52% for black rice varieties. The lowest value of antioxidant activity was observed in white rice. It may be due to reduction in polyphenol content as the concentration of the total soluble phenolic contents was related to lower antioxidant activity (Walter et al. 2013). Jun et al. (2012) evaluated the antioxidant activities and phenolic compounds of pigmented rice (black, red, and green rice) and brown rice brans and reported similar results i.e. black and red rice bran shows high antioxidant activities and contains high amount of phenolic compounds. Indeed, black could be better raw materials for manufacturing the food with high antioxidant activity.

Sensory attributes of black rice *kheer*

Sensory evaluation was done to assess the overall acceptability of the product. The product was prepared under 3 different variations of black rice which was tested by a panel of 20 judges. The sensory parameters were color & appearance, flavor, body & texture and overall acceptability. Table 2, shows that sensory score was found to be highest in the sample K3 that is in the range of 7.7 to 9.80. According to the sensory evaluation the K3 sample containing 20 gram black rice was found to have good color and appearance, taste and flavor and body and texture. The sensory score was in the range of 7.21 to 7.81 of K1 sample followed by K2 sample that has a sensory score ranging from7.38 to 8.54. Considering one attribute at a time the color and appearance was highly scored for sample K3 than K2 than K1. Statistical analysis of sensory data for color and flavor reveals that there is a significant difference in sensory scores of the three black rice *kheer* variants as the p-value <0.05.

Physico-chemical analysis of optimized black rice *kheer*

The data given in Table 3 depicts the proximate composition of black rice *kheer*. Black rice *kheer* contains 58.45±2.10% moisture, 2.84±0.05% ash, 5.11±0.26% fat and 13.54±0.36% protein. Chanu & Yenegi (2015) reported similar composition of raw material and product nutrient contents of black rice i.e. moisture content of rice products prepared from black rice. The fat content of *kheer* (5.11±0.26%) prepared from black rice was found higher than the raw black rice (1.90±0.01%). The similar fat content was reported by Chung & Lim, (1999); Sampong et al. (2011) and Saikia et al. (2012). Protein influences the nutritional quality of rice. The protein content of *kheer* prepared from black rice and raw white rice were 13.54±0.36% and 2.70±1.40%. This may be due to the incorporation of protein rich ingredients specially black rice. Sampong et al. (2011) also observed the protein content of black rice varieties in the range of 8.44% to 10.84%. The amount of ash present in food product plays important role while determining the levels of essential minerals. The ash and crude fiber content

Table 1 Comparison of nutritional profile of black rice and white rice

Particulars	Black rice	White rice
Moisture (%)	11.10 ± 0.04	12.85±0.15
Protein (%)	10.80±0.14	2.70 ±1.40
Fat (%)	1.90±0.01	0.67±0.01
Crude fibre (%)	1.40 ±0.01	0.80±0.08
Ash (%)	2.80± 0.09	1.57±0.04
Zinc(mg/100g)	6.20 ± 0.10	1.38±0.24
Iron (mg/100g)	3.50 ± 0.05	1.20 ±1.00
Total carbohydrate (%)	75.30± 0.12	80.45±0.42
Total phenolic content (mg of GAE/g of dry weight)	75.59^{b}±7.28	11.94^{a}±0.97
DPPH (%)	56.23^{d}±3.56	11.67^{c}±1.18

Values are expressed as Mean± Standard deviation of 3 replications.
Values in the same column with different letters are significantly different at P < 0.05

Table 2 Mean sensory score for *kheer* prepared by using different levels of black rice

Level of black rice Incorporation (%)	Color and Appearance	Body and Texture	Taste and Flavor	Overall Acceptability
K0 (Control)	6.55^{a}±0.09	6.14^{a}±0.07	6.55^{a}±0.05	6.41^{a}±0.07
K1	7.88^{b}±0.12	7.21^{b}±0.09	7.22^{b}±0.04	7.43^{b}±0.08
K2	8.34^{c}±0.11	7.38^{b}±0.13	8.54^{c}±0.08	8.08^{c}±0.11
K3	9.68^{d}±0.11	7.76^{b}±0.05	9.78^{d}±0.07	9.07^{d}±0.07

Values are mean ± Standard Deviation (n=20)
Values in the same column with different letters are significantly different at (.05)

of both the type of *kheer* were at par. The values of crude fiber are comparable with those given by Yodmanee et al. (2011) for different black rice products in the range of 1.63% to 2.06%. Zinc and iron of *kheer* prepared from black rice was found to be differ significantly in comparison to white rice.

Total phenolic content & DPPH activity of optimized black rice *kheer*

Based on evaluation, TPC was found to be 54.46±0.56 and 17.65±0.5413mg gallic acid equivalents/g dry weight for optimized black rice *kheer* and control, respectively. Similarly, 2,2-diphenylpicrylhydrazyl DPPH (%) capacity was found to be 47.90±0.52 and 9.70±0.55 for optimized and control *kheer*, respectively. Therefore, it can be concluded that TPC & DPPH activity of *kheer* prepared from black rice is three times higher than that of control *kheer*. These results were comparable with the observations given by Jun et al. *(*2012) i.e. the phenolic content & DPPH activity of different black rice products ranged from 20.87 to 64.13mg gallic acid equivalents/100g and 49.56% to 11.27%, respectively.

Storage studies of black rice *kheer*

During storage, the pH of black rice *kheer* was decreased and acidity was increased significantly (P<0.05) and their interaction effect was found non-significant on pH and acidity of product (Table 4). Similar results were reported by More et al. 2017. The authors reported that during storage of little millet *kheer* at 6±1°C and the pH decreased significantly while acidity and viscosity increased.

Standard plate count (SPC) count of *kheer* were increased significantly during storage. Yeast & mould count and coliform

Table 3 Chemical composition and antioxidant properties of control and optimized black rice *kheer*

Particulars	Control	Optimized black rice *kheer* (K3)
Moisture (%)	63.59± 1.04	58.45±2.10
Ash (%)	1.81±0.09	2.84±0.05
Protein (%)	9.05±0.14	13.54±0.36
Fat (%)	1.90 ± 0.01	5.11±0.26
Crude Fiber (%)	1.4±0.01	1.51±0.18
Total Carbohydrate (%)	75.3±0.12	72.57±0.51
pH	6.71±0.02	6.81±0.44
Zinc (mg/100g)	1.65±0.05	6.20±0.10
Iron (mg/100g)	0.93±0.05	3.44±0.05
TPC (mg of GAE/g of dry weight)	7.65^{a}±0.54	54.46^{b}±0.56
% DPPH	9.70^{c}±0.55	47.90^{d}±0.52

Values are expressed as Mean± Standard deviation of 3 replications
Values in the same column with different letters are significantly different at P < 0.05

Table 4 Changes in pH, acidity and microbial count of black rice *kheer* during storage

Storage duration (days)	pH	Acidity (%)	SPC (log CFU/g)
0	Control : 6.42±0.32	Control : 0.32±0.01	Control : Nil
	Optimized: 6.40±0.34	Optimized: 0.20±0.12	Optimized: Nil
2	Control : 6.40±0.42	Control : 0.35±0.03	Control : Nil
	Optimized: 6.32±0.53	Optimized: 0.23±0.23	Optimized: Nil
4	Control : 6.31±0.72	Control : 0.41±0.12	Control : 1.45±0.54
	Optimized: 6.30 ±1.01	Optimized: 0.25±0.34	Optimized: 1.32±0.32
6	Control : 6.24±0.71	Control : 0.44±0.03	Control : 2.81±0.16
	Optimized: 6.22±0.50	Optimized: 0.28±0.04	Optimized: 4.13±0.14
8	Control : 6.23±0.22	Control : 0.51±0.06	Control : 3.65±0.41
	Optimized: 6.20±0.11	Optimized: 0.29±0.15	Optimized: 3.42±0.25
10	Control : 6.16±0.64	Control : 0.56±0.02	Control : 4.50±0.23
	Optimized: 6.10±0.45	Optimized: 0.41±0.11	Optimized: 4.40±0.43
12	Control : 6.00±0.56	Control : 0.77±0.06	Control : 6.40±0.42
	Optimized: 5.86±0.86	Optimized: 0.50±0.22	Optimized: 6.89±0.23

Values mentioned as mean ± Standard deviation, (n=3) at (p< 0.05)

Table 5 Cost analysis of functional black rice *kheer* (100g)

Ingredients used	Quantity	Cost (₹)
Black rice	20g	6.4
Coconut Sugar	15g	13.5
Toned Milk	500ml	22.0
Total (Quantity of *kheer*)	300g	41.9
	100g	13.96
Packaging cost	1 cup + Aluminium foil	1.0
	Total	14.96
Processing cost	Per cup	1.0
	Total	15.96
Marketing and distribution expenses	Per cup (100g)	3.29
@25% of product	Total	Rs.19.25

count was found nil during the storage period of 12 days. On 13th day of storage the yeast & mould count were visible. Black rice *kheer* was found to be free from coliform.

Cost analysis

In order to determine the feasibility of this study, cost of production was calculated for optimized *kheer* (Table 5). Cost of raw materials was added along with packaging cost and marketing and distribution expenses. 100g (one cup) of functional black rice *kheer* was prepared approximately ₹ 15.96. The profit margin at 25% of cost of product is also applied which took overall price of black rice *kheer* to a very nominal price of ₹ 19.25.

Conclusions

Antioxidant activity of black rice is two fold stronger with respective to antioxidant activities of blueberries. Black rice can be utilized as a functional food with high antioxidant and low-fat sugar, salt, gluten and cholesterol. It can be used in desserts or dressing or condiment because the black color turns to shiny indigo or purple when cooked. There are limited studies reported on the food product development and functional properties. The research findings of the study can be used to know therapeutic value of black rice when used in different food products.

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RESEARCH ARTICLE

Milk microflora with *S. aureus* alike colony characteristics limits its identification over selective and differential agar media

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Abstract: *Staphylococcus aureus* is considered a significant public health concern, owing to its involvement in food poisoning outbreaks. Milk and milk products are consumed by almost all age groups and serves as a vital nutrition source. Being highly nutritious milk serves as a promising matrix for the proliferation of *S. aureus* and other potential pathogens. Rapid detection of food-borne pathogens like *S. aureus* is vital for ensuring consumer safety. Conventional methods are still considered as the gold standard for pathogen detection in food matrices. Analysis using these methods involves sample plating over selective agar media, followed by microscopic analysis and biochemical tests. Although phenotypic detection based on colour and colony characteristics over selective and differential media is indicative, its sensitivity is compromised by organisms that display colony characteristics similar to the target organism. In this study we attempted to identify organisms from milk matrix showing characteristics similar to *S. aureus* over different agar media that are claimed to be selective for *S. aureus*. Identification of these organisms depicting *S. aureus* alike colony characteristics and development of more selective media can help to expedite *S. aureus* identification from milk and milk products with more confidence.

Abbreviations: *S. aureus*, *Staphylococcus aureus*; BPA, Baird Parker Agar; MSA, Mannitol Salt Agar; VJA, Vogel-Johnson Agar; BHI, Brain Heart Infusion; MALDI-TOF MS, Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry

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Introduction

Food safety is a serious global public health concern, primarily in densely populated areas. *Staphylococcus aureus* is one of the most infamous and widely distributed bacterial pathogens, producing large number of minor skin infections each year, as well as millions of more serious, invasive infections worldwide. Its involvement in food poisoning incidences is attributed to high rate of human skin and nasal carriage, the ability of efficient air-borne spread, and strong survival in fomites. These properties also allow the organism to eradicate competing microorganisms having less ability to endure conditions like elevated temperatures, high osmotic pressure, and relatively low humidity (Le et al. 2021). *S. aureus*, classified as a high-tier priority II pathogen by the World Health Organization is linked to several health problems in both humans and animals. Furthermore, it is one of the most common causes of mastitis in cattle, sheep and goats, resulting in significant reductions in milk output and quality, as well as significant financial losses to dairy industry (Abril et al. 2020). *S. aureus* can gain access to milk either through direct excretion from infected udders with clinical or subclinical mastitis or through contamination from the environment that occurs during the processing of raw milk. Mastitis not only has a negative impact on the production and composition of milk and results in enormous economic losses, but also raises the cost of treatment, decreases the production life span, boosts the rate of elimination and is frequently accompanied by other diseases (Wang et al. 2021). In an earlier study, at least 50% of the dairy farms reported that the entire cost of mastitis were ≤ US$ 1.0/milking cow/day (Vissio et al. 2015). Keeping the above in mind, rapid and accurate identification of *S. aureus* becomes important. For *S. aureus* identification, conventional method includes sample pre-enrichment followed by plating over selective agar media. Commonly used *S. aureus* selective media include Baird Parker Agar (BPA), Vogel-Johnson Agar (VJA), and Mannitol Salt Agar (MSA). Although analysis using these selective media is quite efficient, their performance varies with sample matrices. However, some *S. aureus* alike organisms have been reported to grow well

over these selective and differential media with *S. aureus* alike colony characteristics, making its presumptive identification false and questionable. In this study, we attempted to identify microorganisms from milk matrix having phenotypic characteristics similar to *S. aureus* posing serious challenge to isolation and identification.

Materials and Methods

Sample collection and processing

Raw milk samples including mixed milk (Cattle + Buffalo milk), Goat milk (Beetle Goat), Yak milk, Sheep milk, Camel milk, and Human milk were collected hygienically and transported to laboratory under refrigerated conditions in pre-sterilized glass bottles. Milk samples were serially diluted and appropriate dilutions were spread plated over BPA and VJA for selective pick-up of *S. aureus* alike colonies. Following overnight incubation at 37°C, plates were observed for colonies with similar characteristics to *S. aureus*. The colonies with desirable characteristics (grey-black shiny over BPA and VJA) were selected and processed for further identification.

Bacterial Identification

Individual bacterial colonies with characteristics similar to *S. aureus* were picked up and transferred to Brain Heart Infusion (BHI) broth and incubated overnight at 37°C. The microbial growth from BHI broth was streaked on to BHI agar plates for identification. Individual colonies were subjected to morphological (Gram's staining, Negative staining) and biochemical identification (Catalase and Coagulase) tests (Becker and von Eiff, 2011). Species level identification was carried out by MALDI-ToF-MS facility at College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU). Briefly, pure single colony of test organism from a non-selective agar was smeared onto a spot on MALDI-ToF target plate. Smear was air dried and overlayed with 70% formic acid (1µL) and allowed to dry at room temperature. After drying, the spot was overlayed with HCCA solution (matrix) (1µL) and again allowed to air-dry at room temperature. Target plate was introduced into the biotyper for real-time analysis (Zhu et al. 2015).

Results and Discussion

A total of 6 raw milk samples were used for isolation and identification of organisms showing phenotypic characteristics similar to *S. aureus*. From every milk sample around 50 colonies (25 colonies from each media i.e. BPA and VJA) having characteristics similar to *S. aureus* were picked up and transferred to BHI broth for further analysis. All the isolates showed growth in BHI broth and were streaked on to BHI agar for identification by MALDI-ToF-MS. Apart from *S. aureus*, a total of about 90 isolates fall among six different genera and species *viz*. *Macrococcus caseolyticus*, *Lactococcus lactis*, *Lactococcus garvieae*, *Enterococcus durans*, *Klebsiella pneumoniae* and *Proteus mirabilis* from mixed, yak, goat and camel milk **(Table 1)**. However, all the colonies picked up from sheep and human milk were identified as *S. aureus*. All of the six isolates along with *S. aureus* (ATCC 700698) were analysed for morphological and biochemical properties. Out of 6 isolates, four were found to be Gram positive (*M. caseolyticus*, *L. lactis*, *L. garvieae, E. durans*); while two (*K. pneumoniae*, *P. mirabilis*) turned out to be Gram negative. Later, all the isolates were tested for their catalase and coagulase activity. Among the isolates, *M. caseolyticus*, *K. pneumoniae* and *P. mirabilis* were found to be catalase positive and *L. lactis*, *L. garvieae,* and *E. durans* gave catalase negative reaction. Isolates other than *L. garvieae* and *L. lactis* gave coagulase positive reaction **(Table 1)**. Figure 1 (a-f) presents the colony characteristics of all the target strains alongside *S. aureus* over respective agar media.

This study attempted to identify bacterial strains having colony characteristics similar to *S. aureus* over selective and differential agar media. Milk being a nutritious source for growth and propagation of microbes often contributes to episodes of food-borne infections. Identification and elimination of *S. aureus* alike organisms from milk matrix can help in early pathogen detection and also identification of scenarios like mastitis. In this study,

Table 1 List of isolates used in the study

Lab Identity	Source	Media	Colony characteristics	MALDI-ToF-MS based identification	Microscopic characteristics	Gram's staining	Catalase test	Coagulase test
M3	Mixed milk	BPA	Circular Black center with zone	*Macrococcus caseolyticus*	Cocci, in cluster	+	+	+
M10	Mixed milk	BPA	Shiny black	*Proteus mirabilis*	Rod shaped	-	+	+
C8	Camel Milk	BPA	Circular flat black	*Klebsiella pneumoniae*	Short, plump, straight rod, arranged singly, in pairs, or in short chains and sometimes in clusters	-	+	+
M6	Mixed milk	VJA	Circular flat black	*Lactococcus garvieae*	Cocci, in pairs and short chains	+	-	-
Y1	Yak Milk	VJA	Shiny black	*Enterococcus durans*	Cocci, arranged individually in pairs or short chains	+	-	+
G5	Goat milk	VJA	Pin point small black	*Lactococcus lactis*	Cocci, groups in pairs and short chains	+	-	-

M, Mixed milk; C, Camel; Y, Yak; G, Goat; BPA, Baird Parker Agar; VJA, Vogel Johnson Agar; +, positive reaction; -, negative reaction

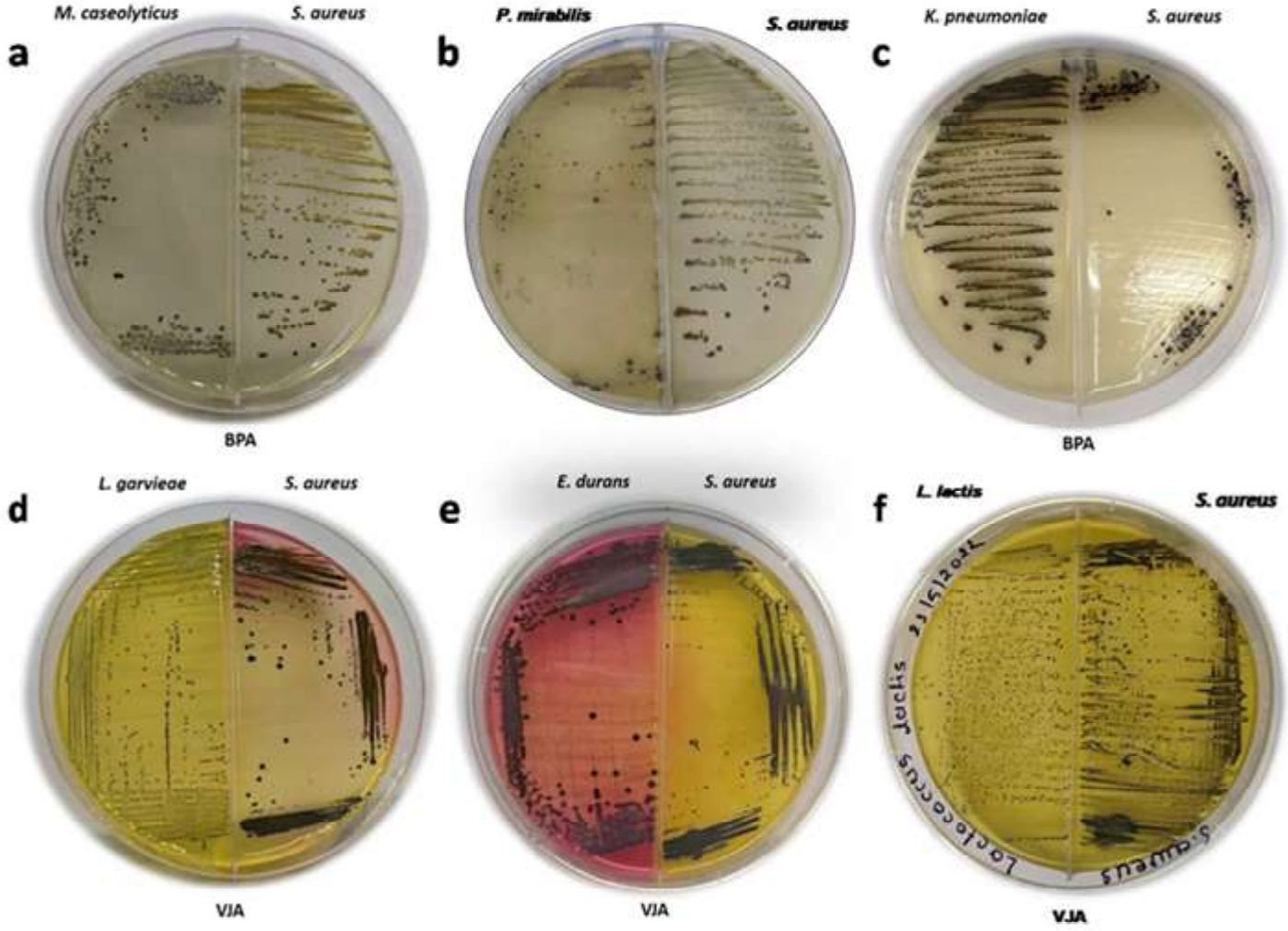

Fig 1. Colony characteristics of *S. aureus* alike strains over BPA and VJA

raw milk samples were processed using standard protocols for enumeration and detection of *S. aureus*. BPA and VJA were used in this study as these two media are considered highly selective for enumeration and detection of *S. aureus* and are recommended by various national and international standards including IS, ISO, BAM, FDA and AFNOR. Earlier, Kim et al., 2010 compared five different selective media and reported BPA and VJA to be highly sensitive and specific media for isolation of *S. aureus* from food matrices. The sensitivity and specificity of BPA was reported as 100% for isolation of *S. aureus* from agriculture products (Kim et al., 2012). After incubation, grey-black shiny colonies from BPA and VJA were selected for further identification. Baird and Lee (1995) described colony characteristics of *S. aureus* over BPA as black colonies with or without halos. The black colony color is attributed to tellurite reduction. From all the samples plated on two different selective media, 300 colonies having similar characteristics like *S. aureus* were isolated and subjected to identification using MALDI-ToF-MS. Even after being represented as selective media for growth of *S. aureus*, 90 non staphylococcal isolates falling under different genera and species were identified. Our results agree with the data presented in oxoid manual which states that organisms other than *S. aureus* can grow on BPA (Oxoid Manual 1998). Similar type of observation of getting different type organisms on BPA was made by Ledina et al. (2018). In this study cheese samples were plated over BPA and the isolates were identified using MALDI-Tof-MS. A large number of non-staphylococcal isolates were identified from BPA including organisms from genus *Macrococcus* and *Enterococcus*. Similarly, in a study targeting *S. aureus* from water samples, majority of the isolates were identified as *Proteus* spp. and *Enterococcus* spp. This study also explains that although Baird-Parker agar is most commonly used to isolate staphylococci, other bacteria known to tolerate tellurite and polymyxin B, can also grow over it (Kaseem et al. 2008). It was reported that organisms from genus *Enterococcus* were selectively isolated on BPA due to their natural resistance against tellurite present in BPA (Vandera et al. 2018). Earlier, Nelson and George (1995) observed the growth of *P. mirabilis*, *Escherichia*

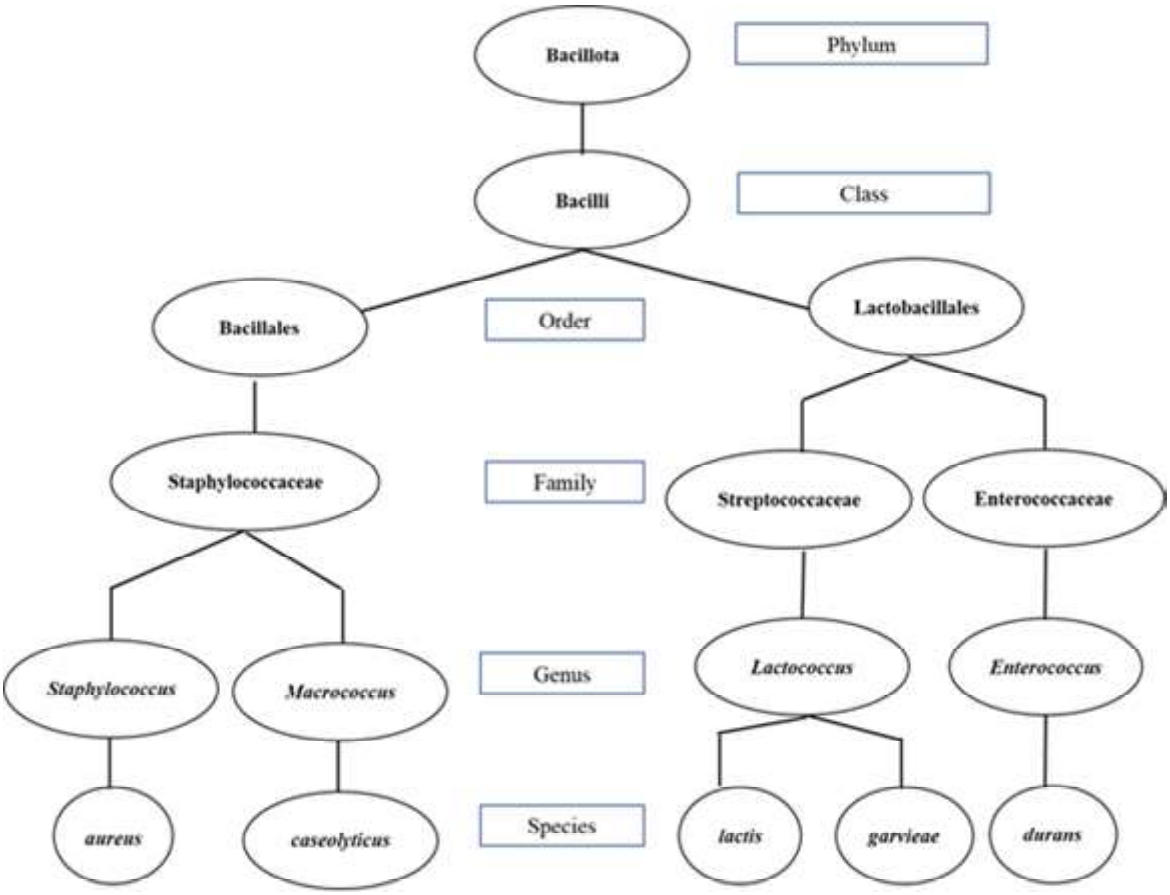

Fig 2. Phylogenetic relationship between test strains

coli and *Enterococcus faecium* over BPA. In our study, among non-staphylococcal bacteria isolated from BPA plates, *M. caseolyticus* was the most abundant. *M. caseolyticus* was formerly classified as *Staphylococcus caseolyticus* and is closely related to staphylococci. *M. caseolyticus* is known to have close evolutionarily relationship with *S. aureus.* Phylogenetic analysis based on 16S and 23S rRNA sequences indicates that the two genera share same ancestor (Tsubakishita et al. 2010). The essential biological pathways of *M. caseolyticus* were also found to be similar to those of staphylococci (Baba et al. 2009). These two microorganisms also share some of the clinically relevant resistance genes and due to presence of resistance genes carrying plasmids it was suggested that horizontal gene transfer may have occurred between *Macrococcus* and *Staphylococcus* species (Chanchaithong et al. 2019; Schwendener et al. 2020). As *M. caseolyticus* is typically considered commensal to livestock, it was also identified in earlier studies in milk samples (Giannino et al. 2009). Only a few reports are available on assessment of selectivity and specificity of VJA and majority of them reported use of this media for growth of *Listeria monocytogenes*. Up to the best of our information, this is the first report showing the growth of *L. lactis*, *L. garvieae* and *E. durans* over VJA from milk matrix.

M. caseolyticus, *L. lactis*, *L. garvieae* and *E. durans* shared colony characteristics with *S. aureus* over selective media and were interestingly identical microscopically. These four strains were cocci arranged in cluster, single or in pairs. *S. aureus* can have different arrangements depending on the source, it can occur as classic bunch like morphology or can occur in tetrad, pair or single cell arrangement. Habib et al. (2015) observed different arrangement of *S. aureus* isolated from different animal sources. The gram-negative isolates (*K. pneumoniae*, *P. mirabilis*) were observed to have rod shaped cells occurring singly or in pairs. *S. aureus* could be distinguished from *K. pneumoniae*, *P. mirabilis, L. lactis*, *L. garvieae* and *E. durans* on the basis of catalase reaction. However, *M. caseolyticus* shared similar morphology, catalase and coagulase reaction with *S. aureus.*

In order to understand the concept behind the utilization of selective components meant for *S. aureus* by other organisms, we analysed the phylogenetic information available for all the organisms as presented in Figure 2. On connecting their evolutionary footsteps, it was observed that four out of six test organisms shared phylogeny with *S. aureus*. The most closely related isolate was *M. caseolyticus*, which share same family with *S. aureus* i.e Staphylococcaceae. Other isolates including *L.*

lactis, *L. garvieae* and *E. durans* share same class Bacilli with *S. aureus*. As clearly evident that *L. lactis* and *L. gravieae* shares same genus so are very closely related to each other. These two organisms are connected to *E. durans* at order level. All of these three isolates belong to order Lactobacillales. All these evolutionary connections indicate that these isolates may have similar biochemical pathways and ability to grow on the selective media specific for *S. aureus*. Two isolates, *K. pneumoniae* and *P. mirabilis* do not share any evolutionary bond with *S. aureus*.

Conclusion

Food poisoning caused by *S. aureus* is a major public health concern around the world and studies have reported contamination of milk and milk-based products with *S. aureus*. In order to deal with the food poisoning cases, accurate identification of causative organisms is very crucial. In this study we focused on *S. aureus* like microorganisms showing similar colony characteristics and growing under similar growth conditions. Identification of these microorganisms is very important to formulate a media with improved selectivity and specificity for *S. aureus* leading to more accurate and rapid conclusive results. Knowledge in this area is steadily improving, but more research is required, not only for improved characterization of *S. aureus* but also to propose new media for inhibiting the *S. aureus* alike strains and to minimize high economic losses caused by *S. aureus* and its toxins to the dairy industry.

Conflict of Interest: None

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RESEARCH ARTICLE

Determination of engineering properties of selected animal feed and fodder materials

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Abstract: The traditional ways of handling feed and fodder in a commercial dairy farm are labour intensive, tiresome and also leads to huge expenses as labour charges. Therefore, there is a need of mechanised feed and fodder distribution unit in order to reduce the human drudgery and other expenses. Thus, an attempt was made to determine the engineering properties (moisture content, bulk density, angle of repose and coefficient of internal friction) of feed which would be affecting the design of a mechanised feed and fodder unit. Three feed materials viz., oat green, wheat straw and concentrate mixture were selected for study. The angle of repose varied from 24.78±0.34 to 37.79±0.76°, the coefficient of friction from 0.187±0.024 to 2.628±1.612 depending on the moisture content and the bulk density of the selected materials. The coefficient of external friction was determined for different fabrication materials including wooden/ply board, galvanized iron, aluminium, mild steel and stainless steel and the maximum coefficient of external friction was offered to the feed materials by ply board followed by mild steel.

Keywords: Animal feed and fodder, Engineering properties, Angle of repose, Coefficient of friction

Introduction

According to the 20th Livestock Census – 2019, India's total livestock population was 535.78 million, up by 4.6% from the previous Census in 2012 (DAHD, 2019). India is the world leader in milk production, although animal productivity is low (1538 kg/year) compared to global average (2238 kg/year), which can be linked to malnutrition due to the huge deficit of animal feed (Vijay et al. 2018). There is no way to sustain cattle husbandry without addressing the challenges of fodder and feed resource development in the country. Due to rising competition between other land uses for cultivable land, further increase in the acreage of fodder crops is not viable (Kumar et al. 2012).

Fodder production in India varies greatly across the country, and its use is determined by cropping pattern, climate, socioeconomic conditions, and the type of cattle. Cattle and buffaloes are often fed fodder from cultivated regions, with collected grasses and top feeds supplementing it to a small extent (Shashikala et al. 2017). Fodder crops are cultivated or harvested for feeding the animals in the form of forage (cut green and fed fresh), silage (preserved under anaerobic conditions) and hay (dehydrated/dried green). Sorghum (2.6 M ha) and Egyptian clover (1.9 M ha) account for approximately 54% of the total cultivated fodder area in the kharif and rabi seasons, respectively (Dagar, 2017). But due to regional and seasonal variation various other crops are cultivated for animal feed purposes. At ICAR-NDRI, Karnal during research trials period oats and wheat were in cropping season and were available to be used as animal feed, hence they were selected for measurement of their engineering properties instead.

Healthy and nutritious feed is important for animal health and productivity. Nowadays, small and marginal dairy farms are no more self-sustaining and/or commercially viable due the huge expenses in terms of labour charges and other maintenance activities. Hence, there is a trend to shift towards large commercial dairy farming practices. In commercial dairy farming, a huge amount of feed is required to be handled. Handling huge quantities of feed manually is tiresome and labour intensive operation in addition to large amounts of feed likely to be wasted during handling. So, it is beneficial to handle the feed materials mechanically instead of opting manual handling. Design and development of feed and fodder handling machines would require a basic knowledge of engineering properties of feed material intended to be handled. Physical and engineering properties are important to understand the behaviour of materials while

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handling, transportation and storage (Puchalski and Brusewitz (1996); Makavana et al. (2018)). The properties like angle of repose, coefficient of internal and external fiction, bulk density and moisture content are some of the important properties which affect conveying characteristics. Seville et al. (1997) explained that the frictional behaviour of biomass grinds in all engineering applications is described by two independent parameters: the coefficient of internal friction, and the coefficient of wall friction. They explained that the coefficient of internal friction determines the stress distribution within particles undergoing strain, and the coefficient of external friction describes the magnitude of the stresses between the particle and the walls of its container. Material properties such as moisture content and particle size affect the frictional properties and densification performance of an individual feedstock (Larsson, 2010).The angle of repose is used in the design of equipment for the processing of particulate solids mainly in the design of an appropriate hopper or silo to store the material, or to size a conveyor belt for transporting the material. The angle of repose is also crucial in correctly calculating the stability of vessels (Bhople et al. 2017).

Engineering properties of feed and fodder materials are key design parameters of storage and handling equipment. Properly designed feed distribution and feeding units help to reduce the losses. As feed material is required to be conveyed while handling, a basic knowledge of how a feed material would behave in certain given conditions could be modelled. Hence, determination of basic engineering properties before design of a successful feed handling system is required. Therefore, an attempt was made to evaluate moisture content, bulk density, angle of repose, coefficient of internal friction and coefficient of external friction for three selected animal feed and fodder materials viz., oat green, wheat straw and concentrate mixture. The research outcome of such studies may be utilised to design and develop feed and fodder handling machines/devices and storage vessels/bins.

Materials and Methods

Raw materials

Green and dry animal feed materials (oat green, wheat straw and concentrate mixture) were selected for the study. Oat (*Avena sativa*) green, wheat (*Triticum*) straw and concentrate mixture (maize grass: 35%; deoiled rice bran: 13%; wheat bran: 12%; groundnut cake: 18%; soy de oiled cake: 19%; mineral mixture: 2% and common salt: 1%) were procured from fodder preparation unit, Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal. Freshly cut oat green & wheat straw and dry powder of concentrate were procured for the study. Oats green and wheat straw harvesting were planned so as to maintain maximum nutrient content in fodder rather than in grains i.e. dough stage to beginning of flowering stage. Oat green and wheat straw were chopped into pieces (average length of 2.5 cm) using a chaff cutter before determining the properties.

Determination of Angle of Repose

Angle of repose of selected feed and fodder material was determined using the developed setup in Research and Development Workshop according to method suggested by Chukwu & Akande (2007) with slight modifications.

A stainless steel 304 sheet of 2 mm thickness was cut in circular geometry with diameter 30 cm to be used as platform for keeping feed material to be evaluated. Steel plate was fixed at the centre of a hopper shaped galvanised iron container of 60 cm external diameter by welding support rods as shown in figure 1. A MS flat with slots at centre and sides was fixed with help of nut and bolt for inserting steel ruler for measurement of height during experiments. Distance between top face of steel plate and top surface of MS flat was measured as 30.7 cm to be used as a constant value for initial height.

The developed setup with outlet duct closure was placed on a levelled platform and proper horizontal level of steel plate was ensured by keeping spirit level on the steel plate. For measuring angle of repose of material, a heap of material was made by slowly pouring the material on the steel plate of the setup. As the heap grew in size, more material was added very carefully on the top of the growing heap till the heap of base diameter covered the full area of the steel plate and the feed material started to slide and fall off from the base plate edge into the outer container. Height of the heap (H_f) was measured by putting MS flat on its place and inserting steel ruler through the centre slot to read value in centimetres. A short video indicating procedure for measurement of angle of repose has been uploaded to YouTube (https://youtu.be/_55t6Fd9mnc). Owing to the non-uniformity of feed material, measurements were done in seven replications for better accuracy. Equation (Eq. 1) as suggested by Sahay and Singh (1996)was used to calculate angle of repose.

$$\theta_r = \left(\frac{H_i - H_f}{D_p}\right) \quad (1)$$

Where,

D_p: Plate Diameter = 30 cm
H_i: Initial Height = 30.7 cm

Determination of Bulk density

For measurement of bulk density a 250 mL beaker was filled with water up to the brim to check full volumetric capacity of selected beaker. It was found to be 300 mL, which was used for calculation of bulk density by ASTM D6683 method (ASTM 2014).

Selected beaker was tared on a precision electronic weighing balance (Model: KERRO BL3003; least count: 1 mg; capacity: 300 g). Feeding material for measurement of bulk density was

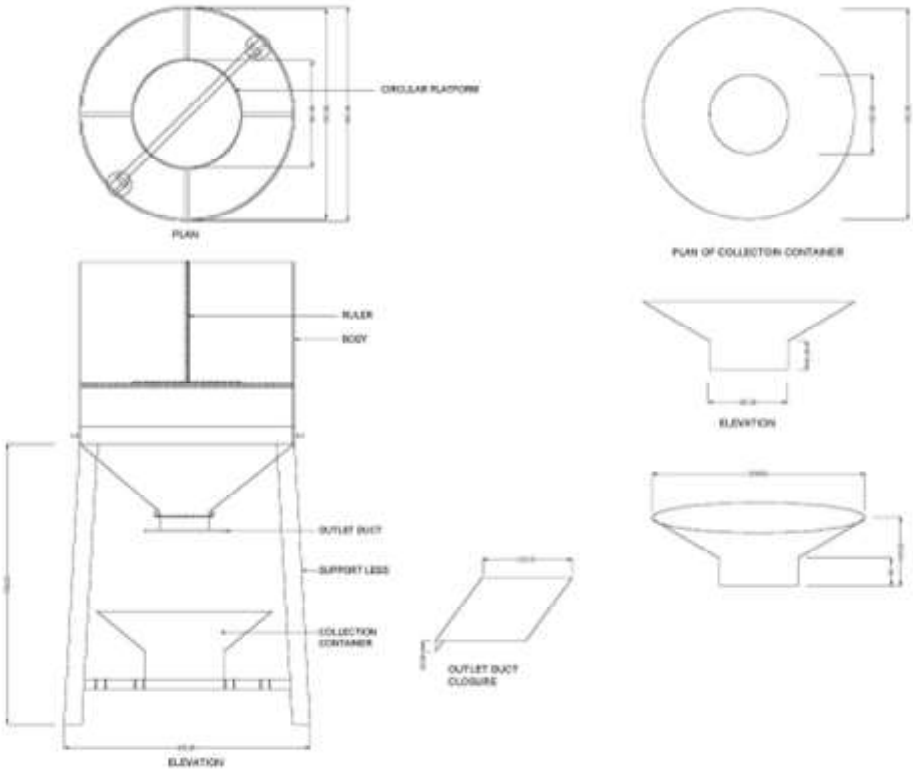

Fig 1. CAD drawing of angle of repose measurement setup

filled in the selected beaker till it overflowed. A spatula was used to gently remove extra material from top to create a flat surface at top of beaker. Weight of the filled beaker was noted. A short video indicating procedure for measurement of bulk density has been uploaded to YouTube (https://youtu.be/_55t6Fd9mnc). Bulk density was calculated by using the following equation (Eq. 2) (ASTM 2014)

$$\rho_{bd} = \frac{W}{V} \qquad (2)$$

Where,

W: Weight of the material, kg
V: Volume of beaker, m^3 (fixed at 300 mL = 0.0003 m^3)

Determination of coefficient of external friction

Coefficient of external friction of selected feed and fodder materials were determined by the method suggested by Sahay and Singh (1996) with slight modifications using the fabricated set-up in the Research and Development Workshop, ICAR – National Dairy Research Institute, Karnal.

A wooden plank was machined on lathe machine to make a hole to fit a container in the hole so that top edge of container would become coplanar with top surface of plank. Container/Pan to be inserted in the hole was cut out from a plastic jar. Wooden plank was cut in shape of rectangle. Plank was provided with four legs of equal height. On one of the shorter side of plank a pulley was provided so that a string could be passed over it. A string of nylon fibre was selected to be used as it offers negligible frictional resistance. A small plastic pan of negligible weight was attached to one side of string using a small aluminium hook of negligible weight. Three cylindrical shaped metal containers without ends of different diameters and heights were chosen and a small slot was made on curved surface so as to pass string through it. Slots on metal containers were meant to adjust string in horizontal position when passing it through pulley hanging over to the pan as shown in figure 2. Five plates of different construction material were chosen and were cut so as to cover central hole in the wooden plank. Five plates were of Stainless Steel (AISI: SS-304), Mild Steel, Aluminium, Galvanised Iron and Ply Board. Selected plates had roughness average value 0.4, 1.5, 1.5, 1.0 µm for SS-304, Mild Steel, Aluminium and Galvanised Iron respectively within range of ± 0.1 µm. Locally available commercial ply board of 6mm thickness was used. Four clamps were made out of stainless steel to hold plates in place during measurements.

For the measurement of coefficient of external friction, the cylindrical shaped metal sample holder was placed on different plate surfaces (mild steel, aluminium, galvanised iron and ply board), which was connected to the weight pan by a nylon string. The weights were gently and carefully put in the pan so that no jerk was observed till the cylinder just started moving from the marked place and total weight in pan was noted as tare reading. Then the sample holder was filled with feeding material so that it

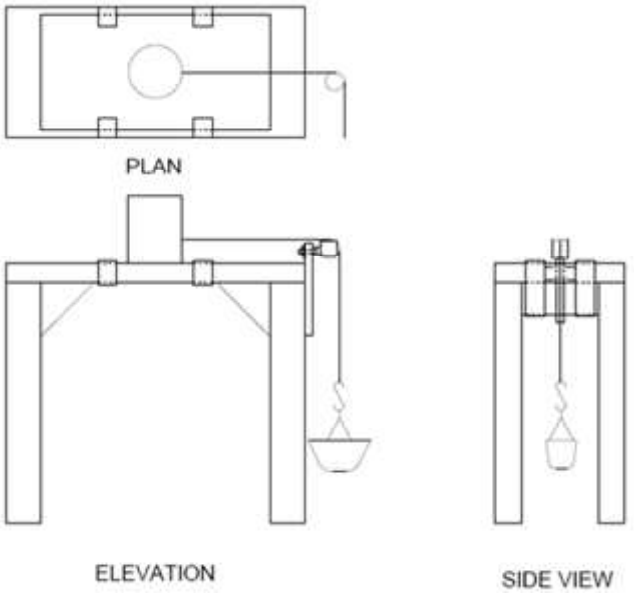

Fig. 2 CAD drawing of angle of repose measurement setup

just overflowed from the top surface. A spatula was used to gently remove extra material from top to create a flat surface at top of container. Extra feed material was removed from the plate surface. Again weights were put in the pan and total weight that caused movement was noted as measured reading. Pulling force was calculated by subtracting tare reading from measured reading. A short video indicating procedure for measurement of coefficient of external friction has been uploaded to YouTube (https://youtu.be/_55t6Fd9mnc). Coefficient of friction was calculated using the following formula (Eq.3). Subramanian and Viswanathan (2007)

$$\mu = \frac{F_p}{N_p} \quad (3)$$

Where,

F_p : weight in pan or pulling force
N_p: weight in pipe or normal reaction

Determination of coefficient of internal friction

Coefficient of internal friction of selected feed and fodder materials were determined by the method suggested by Subramanian and Viswanathan (2007) with slight modifications using the fabricated set-up in the Research and Development Workshop, ICAR – National Dairy Research Institute, Karnal.

The apparatus (as already described in previous section) consisted of one stationary container and one moving cylindrical sample holder. The container inserted in wooden plank was fiilied in with the sample without compactation and levelled off using a scale. The empty sample holder was gently put on the levelled surface of stationery sample container. Then weights were gently and carefully put in the pan so that no jerk was observed till the sample holder just started moving from its place. Total weight was noted as a tare reading. Then the sample holder was filled with the feeding material without compaction and the weights were put in the pan and total weight that caused the movement was noted as measured reading. The filled in sample holder was made to slide on the stationery container with the help of a pulley-nylon string arrangement. Pulling force was calculated by subtracting tare reading from measured reading. A short video indicating procedure for measurement of coefficient of internal friction has been uploaded to YouTube (https://youtu.be/_55t6Fd9mnc). Coefficient of friction was calculated using the following equation (Eq.4). Subramanian and Viswanathan (2007)

$$\mu = \frac{F_i}{N_i} \quad (4)$$

Where,

F_i : weight in pan or pulling force
N_i: weight in pipe or normal reaction

Moisture content

Moisture content was determined gravimetrically using AOAC (1975) method with some modifications. 2-3 g of sample was taken and moisture was removed till the sample obtained a constant weight using a hot air oven kept at 102±2°C.

$$M(\%wb) = \frac{W_1 - W_2}{W} \quad (5)$$

Where,

W : weight of sample
W_1 : weight of pan with sample
W_2 : weight of pan with sample after constant weight

Table 1 Different properties of feed material

Feed material	Moisture content (% wb)*	Bulk density (kg/m^3)	Angle of repose (°)	Coefficient of internal friction
Oat green	64.27±11.45	455.03±11.52	37.79±0.76	0.187±0.024
Wheat Straw	12.07±0.88	40.42±1.76	36.51±1.11	2.628±1.612
Concentrate Mixture	11.74±0.71	504.25±1.82	24.78±0.34	0.357±0.048

*wb: wet basis

Fig. 1 Coefficient of external friction for oat green

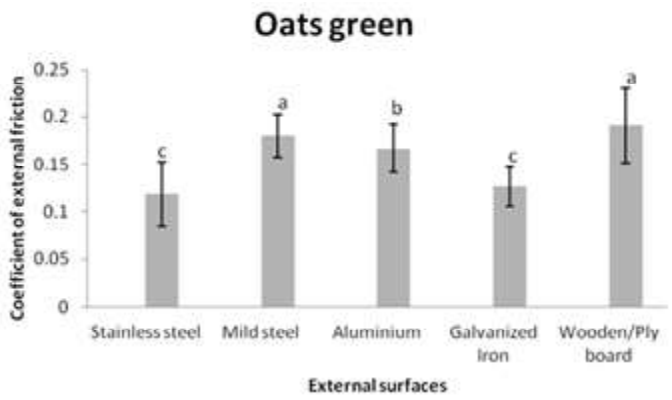

Fig. 3 Coefficient of external friction for oat green

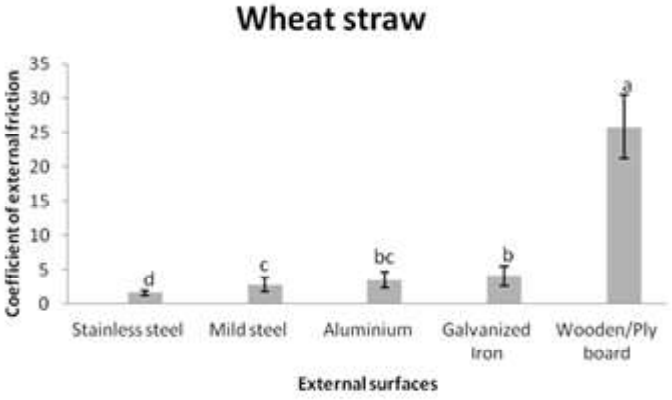

Fig. 4 Coefficient of external friction for wheat straw

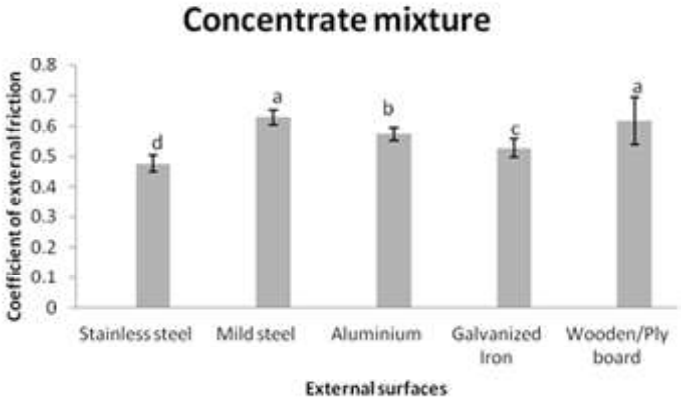

Fig. 5 Coefficient of external friction for concentrate mixture

Statistical analysis

Moisture content, bulk density and angle of repose trials were replicated seven times and coefficients of internal and external friction values were taken in seven replicates at four different positions with two sample holders (56 effective replicates). Statistical analysis was performed using WASP- Web Agri Stat Package 2.0 of ICAR-Central Coastal Agricultural Research Institute, Goa.

Results and Discussion

Physical and engineering properties including moisture content (%wb), bulk density, angle of repose, coefficient of internal and external friction of various animal feed materials were determined.

Angle of repose

Angle of repose of the selected feed materials was estimated using the developed angle of repose set up. Highest angle of repose value was observed for oat green (37.79±0.76°) and lowest angle of repose was observed for concentrate mixture (24.78±0.34°). Even though wheat straw was in dry form, it showed an almost similar angle of repose value (36.51±1.11°) of oat green. Makavana et al. (2018) had conducted studies on wheat straw and found similar values of angle of repose (38.23°). Bhople et al. (2017) carried out a study to understand the effect of moisture content on angle of repose for different cereals and pulses and they conclude that the angle of repose increased as moisture content of the grains increased. This reported finding is at par with the present study.

Bulk Density

The bulk density of oat green was 455.03±11.52 kg/m^3. McNulty and Kennedy (1982) reported the bulk density value of oat green to be 439 kg/m^3 and the current observation was at par with their finding. Bulk density of wheat straw was found to be 40.42±1.76 kg/m^3, which was in accordance with the observations of Guo et al. (2013). Lam et al. (2007) carried out a study to analyse the bulk and specific density of wet and dry wheat straw and they inferred that the bulk density increased with moisture content of particles. Highest bulk density among the tested samples was observed for concentrate mixture (504.25±1.82 kg/m^3). Bahnasawy and Mostafa (2011) had conducted studies on feed pellets and they reported the bulk density value of pellets as 640 kg/m^3. Thus, the values obtained in the current study were in corroboration with literature reported data. Tang et al. (2014) established a relation between moisture content and bulk density of biomass materials. According to them, the volume of the biomass will be larger if it has more moisture in it and therefore, the bulk density of biomass will be lower.

Coefficient of external friction

Coefficient of external friction between the fodder materials and surfaces of different fabrication material viz., stainless steel, mild steel, aluminium, galvanized iron and wooden/ply board were determined. Friction between stainless steel and all the three fodder materials were least whereas wooden/plywood offered highest resistance or friction to the fodder materials oat green

(Fig. 3) and wheat straw (Fig. 4). Friction between mild steel and concentre mixture were highest while analysing the coefficient external friction of concentrate mixture (Fig. 5). Askari et al. (2017) observed and reported that the coefficient of external friction was influenced by product variety, grain moisture content, the material of contact surface, sliding velocity etc.

Coefficient of external friction between the selected feed materials with different surfaces including stainless steel, mild steel, aluminium, galvanized iron and wooden/ply board were determined. It was observed that the coefficient of external friction between oat green with mild steel and ply board were statistically similar and these surfaces offered highest friction. Whereas, stainless steel and galvanized iron surfaces offered least friction to oat green and those were statistically similar.

Coefficient of external friction between wheat straw with different surfaces were observed to be statistically significant. Wooden/ ply board offered highest friction to wheat straw (25.88) whereas stainless steel offered the least friction (1.585). Frictional resistance between wheat straw with aluminium and galvanized iron were statistically similar. Aluminum and mild steel also showed statistically similar values of coefficient of external friction to wheat straw.

Wooden/plywood and mild steel offered the highest values of coefficient of external friction to concentrate mixture and stainless steel offered the least.

Coefficient of internal friction

Coefficient of internal friction is an important design parameter for storage and distribution of cattle feed materials. The values of coefficient of internal friction for oat green, wheat straw and concentrate mixture were 0.187±0.024, 2.628±1.612 and 0.357±0.048, respectively. The reported range of coefficient of internal friction of wheat straw was 0.765-1.586 by Chevanan et al. (2008). There was observed a significant increase in the estimated coefficient of internal friction value of wheat straw. Also, there observed no correlation between moisture content and coefficient of internal friction of the selected materials. Mani et al. (2004) also reported that the adhesion coefficient did not exhibit dependence on moisture content of corn stover.

Moisture Content

Table 1 illustrates the values of moisture content (%wb), bulk density, angle of repose and coefficient of internal friction of selected feed materials. A significant deviation was observed in the values of moisture content for oat green during different trials with an average value of 64.27±11.45 (%wb). Gill and Omokanye (2018) studied the "Potential of Spring Barley, Oat and Triticale Intercrops with Field Peas for Forage Production, Nutrition Quality and Beef Cattle Diet" and reported the moisture content range of green fodder as 55-77 (%wb). Therefore, the observed moisture content of oat green was in concordance with the earlier findings. The average moisture content of wheat straw was 12.07±0.88 (%wb). Guo et al. (2013) conducted a study on the physio-chemical properties of wheat straw and reported the moisture content value of wheat straw as 12.8 (%wb). Hence the observed moisture content value was at par with the literature value. The concentrated mixture had an average moisture content of 11.74±0.71(%wb). Alengadan et al. (2013) studied the moisture content control during cattle feed production and they reported that the moisture content of cattle feed should not be more than 11.5% and the excess moisture content in cattle feed results in serious quality problems. In another study conducted by Bahnasawy and Mostafa (2011), the reported moisture content values for feed pellets was 16-17 (%wb).

Conclusion

Engineering properties of cattle feed materials play a key role in the design of a mechanised feed and fodder handling and distribution system. Therefore, important feed properties like moisture content, bulk density, angle of repose, coefficient of internal and external friction were determined for the selected feed materials viz. oat green, wheat straw and concentrate mixture. Laboratory scale equipments were developed for determining angle of repose and coefficient of friction. Moisture content and bulk density of the selected materials ranged from 11-65% and 40-500 kg/m^3, respectively. Angle of repose and coefficient of internal friction; which play the key role in storage and distribution equipment design, ranged between 24-37° and 0.18 - 2.6, respectively. These insights into the feed properties will help to choose the appropriate material for fabrication as well as for the overall design and development of mechanised feed handling and distribution units. These data could be helpful in selection of material for fabrication of equipment.

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RESEARCH ARTICLE

Impact of seasonal variation on endocrine profile during an estrous cycle in Jersey crossbred dairy cows

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Abstract: The objective of the study was to investigate the influence of season *viz.*, summer, winter, and isothermic (spring and autumn season), on endocrine profile in dairy cows. The daily temperature-humidity index (THI) was recorded using a dry and wet-bulb thermometer to envisage its variation among different seasons. For analysis of endocrine profile, blood samples were collected from thirty dairy cows (n=10 in each season) at 5-day interval i.e. day 0 (day of estrus) to day 20, of the estrous cycle for ELISA based estimation of estradiol-17β, progesterone, and cortisol. In results, the THI varied significantly ($p<0.01$) in different seasons with maximum THI in the summer (72.54), followed by the isothermic (64.7) and lowest in the winter season (54.12). Among the endocrine profile, the plasma cortisol levels had a significant variation ($p<0.05$) in different seasons with increased levels in the winter followed by the summer season, however, differences were non-significant ($p>0.05$) in plasma progesterone and estradiol-17β. In summary, seasonal variation barely led to significant changes in the reproductive hormones (estrogen and progesterone) but alleviated cortisol levels during summer and winter season had been suggestive of stress and could result in reduced fertility.

Keywords: Cortisol; Estradiol-17β; Progesterone; Temperature-humidity index

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Introduction

Dairy cows living in tropical climates are subjected to severe weather conditions including extended periods of high ambient temperature and sun radiation. High ambient temperature also affects the reproductive hormone profiles of cattle. The endocrine system is involved in animal adaptation to stress and it has a significant impact on circulating hormones (Johnson and Vanjonack 1976). Because of the neuroendocrine response to climatic variables, climatic factors or seasonal fluctuations have a significant impact on animal behavior, impacting animal production and health (Sejian et al. 2013). The pituitary, thyroid, and adrenal glands play an important role in acclimatizing dairy cattle to environmental stress through various thermoregulatory and metabolic functions (Johnson et al. 1988). Cows under heat stress secrete higher levels of progesterone. The adrenal gland acts as a source of this progesterone. This level of progesterone inhibits the LH surge of estrus and prevents ovulation (Nazafi et al. 2003).

There is a reduction in the concentrations of estrogen and inhibin from the dominant follicle which results in the inhibition of pituitary FSH secretion. Summer had lower levels of estradiol in follicular fluid than winter while comparing the first-wave dominant follicles on day 7 of the cycle (Wolfenson et al. 1997). Cortisol, prolactin, and thyroxin are other hormones whose levels are altered during different seasonal conditions. Activation of the hypothalamo-pituitary-adrenal axis during stress reduces the pulsatility of GnRH/LH by actions at both the hypothalamus and pituitary gland, ultimately depriving the ovarian follicle of adequate LH support (Phogat et al. 1997), thus, the cow fails to maintain estrous cycles and consequently lead to anestrous. This result in the loss of integrity of the granulosa cells and subsequently the oocyte competence, although estrus and fertilization may occur, the conceptus fails to develop into a pregnancy (Dobson and Smith 2000). Keeping in view these aspects, the objective of study was to investigate the seasonal effects *viz*., summer, winter, and isothermic (spring and autumn season) seasons, on endocrine profile in dairy cows.

Materials and Methods

This study was conducted at Livestock Farm Complex, CSKHPKV, Palampur (Zone-2 of agroclimatic zones of Himachal Pradesh; Mid-hills and sub-humid zone), from May 2021 to April 2022 for a period of twelve months comprising three seasons including summer (May to August), winter (November to February), and isothermic season i.e. spring (March and April) and autumn (September and October) season. The daily mean values of wet and dry bulb temperatures at 12:00 noon were recorded from a wet and dry bulb thermometer placed outside the animal shed. THI was calculated according to the equation reported by NRC

$$THI = 0.72 \times (Tdb + Wdb)\,°C + 40.6$$

Tdb = Dry bulb temperature (°C)

Wdb = Wet bulb temperature (°C)

Thirty cows (Jersey crossbred; n=10 in each season) kept under standard feeding conditions, under natural light, housed in dry concrete sheds, and milked twice daily were selected for the study. Cows were clinically healthy and normal cyclic, with no history of reproductive abnormality. Blood samples were collected from the jugular vein, on day 0 (day of estrus), 5, 10, 15, and day 20 of the estrous cycle in each season from all the animals for estimation of hormonal (estradiol-17β, progesterone, and cortisol) attributes. From each cow, 10 ml of blood was collected in a heparinized vial and centrifuged at 3000 rpm for 10 minutes for the separation of plasma. The plasma samples were stored at -20°C until further analysis.

All the hormones were estimated from stored plasma samples (-20°C) after thawing them at room temperature. ELISA kits were used to analyze different hormones. Progesterone was estimated using DRG instrument GmBH, Germany ELISA kit, estradiol-17β using BT LAB Bovine Estradiol ELISA kit, and cortisol using BT LAB Bovine Cortisol ELISA kit. The estimations were carried out in TECAN SUNRISE Microplate Absorbance Reader (TECAN Austria GmbH, Austria) present in the Department of Veterinary Gynaecology and Obstetrics.

The recorded data were statistically analyzed using one-way ANOVA and with NCSS 2020, USA (Version 22.0.4) software.

Results and Discussion

In our study, the temperature-humidity index was significantly different ($p<0.01$) in different seasons with the summer season having a higher THI followed by the isothermic and winter seasons (Table 1). The temperature-humidity index varied significantly in the three seasons but not up to an extent that would result in heat stress in cattle (Morton et al. 2007; Schuller et al. 2014).

A significantly higher ($p<0.01$-0.05) value of plasma cortisol was recorded during winter as compared to the summer and isothermic seasons on days 10, 15, and 20 of the estrous cycle (Table 2). These findings were in agreement with Titto et al. (2012) who reported a significant increase in plasma cortisol levels during winter in dairy cows. On the contrary, Dobson and Smith (2000) and Ahmed and Abdalla (2012) reported a rise in plasma cortisol levels in the summer season in dairy cows as compared to the

Table 1: Average values of dry bulb temperature, wet bulb temperature, and the temperature-humidity index (THI) during summer, winter and isothermic season (Mean ± S.E)

Season	Dry bulb temp. (°C)	Wet bulb temp. (°C)	THI
Summer	26.25 ± 1.0^{x}(24.06-27.96)	18.11 ± 1.1^{a}(14.96-19.87)	72.54 ± 0.11^{x}
Winter	14.82 ± 1.31^{y}(13.96-18.36)	3.95 ± 1.5^{c}(0.93-8.03)	54.12 ± 0.34^{z}
Isothermic	21.96 ± 1.22^{x}(18.58-23.83)	11.5 ± 2.01^{b}(6.8-16.6)	64.7 ± 0.38^{y}

^{x,y,z} Values with superscripts in same column differ significantly ($p\leq0.01$)

^{a,b,c} Values with superscripts in same column differ significantly ($p\leq0.05$)

Table 2 Values of plasma cortisol (ng/ml) in dairy cows at different days of estrous cycle during summer, winter and isothermic season (N=30) (Mean ± S.E)

Day of estrous cycle	Summer	Winter	Isothermic
Day 0	4.17 ± 0.61	4.52 ± 1.23	3.46 ± 0.89
Day 5	4.16 ± 0.35	6.02 ± 2.51	2.62 ± 0.22
Day 10	3.76 ± 0.41^{y}	5.60 ± 0.75^{x}	2.20 ± 0.35^{y}
Day 15	4.35 ± 0.40^{b}	5.77 ± 0.92^{a}	2.69 ± 0.47^{b}
Day 20	4.34 ± 0.42^{b}	5.09 ± 0.92^{a}	1.94 ± 0.1^{b}

^{a,b,c} Values with superscripts in same row differ significantly ($p\leq0.05$)

^{x,y,z} Values with superscripts in same row differ significantly ($p\leq0.01$)

Table 3: Plasma progesterone (ng/ml) concentrations in dairy cows at different days of estrous cycle during summer, winter and isothermic season (N=30) (Mean ± S.E)

Day of estrous cycle	Summer	Winter	Isothermic
Day 0	0.48 ± 0.1	0.49 ± 0.13	0.46 ± 0.03
Day 5	1.96 ± 0.38	1.90 ± 0.18	2.56 ± 0.41
Day 10	4.02 ± 0.43	4.33 ± 0.83	4.78 ± 0.62
Day 15	3.58 ± 0.46	4.00 ± 0.34	4.97 ± 0.6
Day 20	0.95 ± 0.14	0.83 ± 0.16	0.70 ± 0.09

Table 4: Plasma estradiol-17β (pg/ml) concentrations in dairy cows at different days of estrous cycle during summer, winter and isothermic season (N=30) (Mean ± S.E)

Day of estrous cycle	Summer	Winter	Isothermic
Day 0	4.3 ± 0.05	3.67 ± 0.88	5.59 ± 1.32
Day 5	2.46 ± 0.33	2.36 ± 0.46	2.44 ± 0.04
Day 10	2.65 ± 0.48	2.47 ± 0.67	2.85 ± 0.34
Day 15	2.52 ± 0.52	2.81 ± 0.45	2.91 ± 0.22

winter and rainy seasons. This variation noted by different workers may be due to the climatic conditions prevalent in their area of work.

The cyclic pattern of plasma progesterone concentration recorded in our study (Table 3) was in agreement with known changes in corpus luteum function in cows that occur during the estrous cycle (Schomberg et al. 1967; Hafez 2008).

The plasma progesterone concentration recorded in our study was lowest at day 0 (day of estrous), reaches a peak at the mid-luteal phase, and then starts to decrease with declining corpus luteum and was in agreement with Hafez (2008). However, no significant difference was recorded for plasma progesterone concentrations in different seasons (Table 3).

The peak levels of estrogen were achieved during the last part of the estrous cycle which is during proestrus which may be due to the presence of preovulatory follicle development (Alvarez et al. 2000; Hafez 2008; Naik et al. 2013).

During the start of the estrous cycle, in the phase of estrus, the estrogen concentration starts decreasing significantly from day 0 (day of estrus) to day 3 of the estrous cycle and causes ovulation in metestrus and initiates CL formation (Hafez 2008; Naik et al. 2013; Noakes et al. 2019). Later on, from the 3rd to 6th day, the concentration of estrogen increases significantly which is due to the first dominant follicle development (Alvarez et al. 2000; Naik et al. 2013). But after 6th day of the estrous cycle, the concentration decreases abruptly indicating dominant follicle atresia, developed during the first follicular wave (Hafez 2008; Noakes et al. 2019). However, in our study, no significant difference ($p>0.05$) was recorded between the plasma estradiol-17β concentration during summer, winter, and isothermic seasons (Table 4).

Conclusions

Dairy cows were more prone to cold stress as compared to heat stress as increased values of plasma cortisol were recorded in the winter season followed by the summer season. In peroration, the seasonal variation barely led to significant changes in the ovarian steroid hormones (estrogen and progesterone), however, alleviated cortisol levels during summer and winter season had been suggestive of stress and could result in reduced fertility in the area under study.

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RESEARCH ARTICLE

Influence of supplementary nickel on feed intake, nutrient utilization and growth performance in Murrah buffalo calves

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Abstract: Eighteen male Murrah buffalo calves of about similar age (8.7 months) and body weight (125 kg) were selected from Livestock Research Centre, ICAR- National Dairy Research Institute, Karnal, Haryana, India and divided into 3 groups of 6 animals using randomised block design to investigate the effect of different levels of nickel (Ni) supplementation on feed intake, nutrient utilization, growth performance and rumen fermentation parameter. All the animals were fed to meet their nutrient requirements ICAR (2013), however, the animals in groups T_1, T_2 and T_3 were supplemented with 0, 5 and 10 ppm of Ni, respectively. Daily DM intake and fortnightly body weights were recorded in the morning before offering feed and water. Rumen liquor samples were collected at 0, 60 and 120 days of the experiment to analyze different rumen fermentation variables. Different levels of Ni in the diet did not influence nutrient intake, digestibility of nutrients (DM, OM, CP, EE, NDF and ADF), nitrogen balance, growth rate and feed conversion ratio. The values of various rumen fermentation parameters like pH, total volatile fatty acids, ammonia-N and TCA precipitable-N were similar in the three groups. The urease activity in rumen liquor was the highest ($P<0.05$) in group T_3. The propionate level increased ($P<0.05$) while that of butyrate decreased in group T_3 as compared to groups T_1 and T_2 showing no significant effect on acetate concentration. Hence, supplementation of Ni upto 10 ppm in the ration did not affectnutrient utiliisation and growth performance in male Murrah buffalo calves, however, urease activity and proportion of propionate increased to a greater extent in calves supplemented with 10 ppm Ni.

Keywords: Buffalo calves, Growth performance, Nickel, Nutrient digestibility, Rumen fermentation

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Introduction

Nickel (Ni) is a possible essential trace element (NRC, 2005). Nickel is an integral part of the soil, plants and water. Nickel concentration in plants is affected by several factors including plant species, stage of maturity, pH of the soiletc.(Underwood, 1977; Sapek and Sapek, 1980). Faecal excretion is the major route for eliminating unabsorbed Ni and urinary excretion is the major route for eliminating absorbed Ni (Von, 1997). Nickel is an important element for the biosynthesis of hydrogenase, carbon monoxide dehydrogenase enzyme (Can et al. 2014) and discovered in several genera of bacteria in the rumen. Nickel-Fe hydrogenase enzyme was required for the growth of anaerobic *Enterobacteriaceae* and this bacterium was required for vitamin B_{12} synthesis in the rumen. Cellular Ni was found to be localized in the nucleic acid fraction (Wacker and Vallee,1959; Sunderman, 1965) and contribute to the stability of RNA, DNA or ribosome. Nickel might have a function in nucleic acid and protein metabolism. Rumen bacterial urease (EC3.5.1.5) is a Ni -dependent enzyme (Hausinger, 1986). The addition of Ni in the diet increased the recycling of nitrogen in the rumen by increasing ruminal epithelium urease activity (Spears and Hatfield, 1980). Nickel with marginal or adequate protein diets containing urea was fed to ruminants and a constant increase in rumen ammonia concentration and serum urea was observed (Spears and Hatfield, 1978). Nickel is also an important element for adequate immune system in the animal body. Alteration in Ni concentration of diet affects the production and action of some hormones like insulin, growth hormone, prolactin, thyroid hormone, noradrenaline, adrenaline and aldosterone (La Bella et al. 1973). The normal dietary requirement of Ni for domestic animals has not been well established, however, Ni requirement was higher for ruminants than non-ruminants which was considered to be in the range of 300 to 350 µg/kg dietary DM. The maximum tolerable level of Ni in the diet of cattle and sheep should be less than 100 ppm while for chicks and pigs it is 250 ppm. The definite role (s) of Ni in animal metabolism

remains a mystery. Hence, the present study was undertaken to find out the influence of supplementary Ni on feed intake, nutrient utilization, growth performance and rumen fermentation parameters in Murrah buffalo calves.

Materials and Methods

Animals, diets and experimental design

The study was conducted at Livestock Research Center of ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana, India. The experimental protocol was approved by the NDRI-Institutional Animal Ethics Committee (IAEC) under the IAEC approval No. 45-IAEC-19-1 governed by the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA, Govt. of India).

Eighteen male Murrah buffalo calves were distributed randomly into 3 groups of 6 animals each based on body weight (125±9.67 kg) and age (8.70±0.86 months) using randomised block design. Deworming was done before the start of the animal trial. All the animals were fed to meet their nutrient requirements as per ICAR (2013), however, experimental animalsreceived a basal diet along with Ni (in form of nickel sulphate hexahydrate) @ 0, 5 and 10 mg/kg DMI in groups T_1, T_2 and T_3, respectively for a period of 120 days.

Oats fodder and concentrate mixture were supplied in the ratio of 60: 40 (on DM basis) in ration to meet the requirements as per ICAR (2013) standards**.** The animals were given fresh and clean water free of choice twice daily at 09.00 h and 15.30 h. All the animals were housed in individual pens and clean surroundings were ensured throughout the experimental period.

Body weight, DM intake, average daily gainxand feed conversion ratio

The body weight of animals was measured at fortnightly intervals. The DMI was measured based on body weight and expected gains. The average daily gain (ADG) and feed conversion ratio (FCR; kg DM intake/kg BW gain) were calculated.

Metabolic trial and chemical composition of biological samples

A metabolic trial was conducted at the end of growth trial during which representative samples of different feeds offered (Oats fodder and concentrate mixture) and residues were collected and dried in hot air oven at 65°C till a constant weight was attained. Dried samples were ground to pass through 1 mm sieve size and stored in air tight containers. Feeds, residues and faeces were analysed for proximate principles viz. DM, OM, CP, EE and total ash (AOAC 2005) and cell wall constituents (NDF and ADF) as per Van Soest et al. (1991). Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) in feeds were determined as Licitra et al. (1996). Total digestible nutrient (% TDN) was calculated (NRC, 2001). The Ni contents in a feeds and water samples were measured by atomic absorption spectrophotometer (ZEEnit700P) at ICAR- Central Soil Salinity Research Institute, Karnal, Haryana.

Rumen fermentation parameters

Rumen liquor samples were collected at 0, 60 and 120 days of the feeding trial before feeding and watering from four animals for each group using a stomach tube attached to a vacuum pump. Rumen liquor was strained through four layers of cheesecloth into plastic containers and kept in -20°c for further analysis. Rumen pH and urease activity were measured immediately after the collection of rumen liquor using a digital pH meter (Weatherburn,1967).

Ammonia-N and TCA precipitable-N were measured using micro Kjeldahl method (KELPLUS-CLASSIC-DX). The concentration of total volatile fatty acids (TVFA) was estimated (Barnett and Reid 1956). The individual fatty acid (IVFA) levels were measured by gas chromatograph (NUCON-5700) as per Cottyn and Boucque (1968).

Statistical analysis

The data were analysed using Statistical Package for Social Sciences (SPSS, V21.0; Inc., USA).

Results and Discussion

Feed intake and growth performance

The chemical composition of feeds has been shown in Table 1. The Ni content in basal diet was 1.67 mg/kg DM. Dietary Ni supplementation up to 10 ppm did not affect feed intake, fortnightly body weight, FCR and ADG of Murrah buffalo calves (Table 2). Spears and Hatfield (1978) found that early weaned lambs fed a semi-purified diet containing 0.065 ppm Ni showed

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

Parameter	Oats fodder	Concentrate mixture
Nutrient composition		
DM	32.23	92.32
OM	90.09	88.73
CP	8.02	19.22
TA	9.91	11.27
EE	2.77	4.81
NDF	57.39	26.93
ADF	44.75	15.68
NDICP	2.82	2.74
ADICP	1.34	0.89
TDN*	60.79	75.25
Ni (ppm)	1.50	1.97

*Calculated value (NRC, 2001)

gains similar to animals receiving Ni 5 ppm supplemented and observed no outward deficiency symptoms. O'Dell et al. (1970) found significantly improved FCR due to addition of Ni in the diet of calves compared to the non-supplemental group. Spears et al. (1979) reported that supplementation of Ni @ 5 ppm with high energy and low protein diet fed to steers and improved FCR compared to the control group. Singh et al. (2018) reported that the heifers receiving a diet containing 3 ppm Ni showed significantly improved feed intake weight gain and FCR as compared to those receiving 1.5 ppm Ni and the non-supplemented groups. Anke et al.(1977) suggested that supplementation of 5 ppm Ni along with basal diet-fed goats showed 21% higher weight gain than those fed a basal diet with 1 ppm Ni. Bersenyi et al. (2004) observed that dietary supplementation of 50 mg Ni/kg DM intake fed to broiler chicken showed a slight improvement in body weight gain and feed conversion efficiency while Ni added at a level of 500 mg/kg in broiler chick diets reduced BW gain by 10% as compared to control group and resulted in poor FCE. Kirchgessner and Roth (1977) found that supplementation of Ni at the level of 125, 250 and 375 ppm had no effect on feed intake and growth performance while at 500 ppm level Ni reduced feed intake and growth rate in pigs. Oscar et al. (1987) reported higher body weight gain and improved feed efficiency in the diet of steers fed 5 ppm Ni.

Plane of nutrition, digestibility of nutrients and nitrogen balance

The digestibility of nutrients (DM, OM, CP, EE, NDF and ADF) was similar in all groups (Table 3). Nitrogen utilisation was not affected by supplementation of Ni up to 10 ppm in the diet. The Ni supplementation at a level of 5 or 10 ppm in the diet did not affect plane of nutrition in Murrah buffalo calves (Tabel 4). O'Dell et al. (1970) reported no significant change in the apparent digestibility of DM, CP, NFE and gross energy in dairy calves by the addition of Ni to the diet. Paula et al. (2005) reported that supplementation of Ni at 5 ppm with a low protein (3.1% CP) the diet of male sheep showed no effect on the apparent digestibility of DM, CP, OM and nitrogen balance while receiving high protein (8.2% CP) diet with Ni supplementation @ 5 ppm reduced digestible DM, digestible energy and metabolizable energy as compared to the non-supplemented group.

Table 2 Effect of Ni supplementation on feed intake and growth performance

Parameter	Day	Group			SEM	*p*-value
		T_1	T_2	T_3		
Body weight (kg)	0	126.25	125.65	123.97	6.12	0.99
	120	209.02	207.93	210.24	8.57	0.97
	Mean	166.14	164.36	164.68	3.38	0.90
ADG (g)		689.75	685.63	718.88	20.97	0.55
DM intake (kg/d)		4.85	4.87	4.88	0.07	0.90
DM intake (kg/100 kg BW)		2.83	2.88	2.88	0.02	0.58
FCR (kg feed intake/kg BW gain)		7.03	7.13	6.88	0.20	0.76

Table 3 Effect of Ni supplementation on nutrient digestibility (%) and nitrogen balance

Parameter	Group			SEM	*p*-value
.	T_1	T_2	T_3		
Digestibility (%)					
DM	58.49	59.72	61.63	0.66	0.15
OM	60.63	61.21	62.99	0.62	0.30
CP	66.33	66.65	66.70	0.82	0.98
EE	83.29	82.66	83.99	0.63	0.73
NDF	51.33	51.47	53.23	1.49	0.87
ADF	39.85	38.86	42.05	1.00	0.46
Nitrogen balance					
N intake (g/d)	104.73	100.71	106.89	1.33	0.16
N excreted in faeces (g/d)	35.26	33.59	35.60	1.06	0.75
N excreted in urine (g/d)	41.06	40.08	42.22	1.13	0.84
Total N outgo (g/d)	76.32	73.67	77.82	2.54	0.82
Absorbed N (g/d)	69.47	67.12	71.29	1.02	0.27
N absorbed (% N intake)	66.33	66.65	66.69	0.82	0.98
N balance (g/d)	28.41	27.04	29.07	1.67	0.89
N retention (% N intake)	27.36	26.91	27.36	1.79	0.99

Nitrogen absorption and excretory patterns were not affected by Ni supplementation. Spears and Hatfield (1978) found no change in faecal N by supplementation of Ni in the diet of lamb while urinary N excretion was significantly higher in the group of lambs receiving a low Ni diet as compared to the 5 ppm Ni-supplemented group indicating that Ni plays a vital role in protein metabolism while no difference in nitrogen excretion was observed between the low and adequate Ni group in the second collection period conducted at 56 days.

Rumen fermentation parameters

Average values of pH, total volatile fatty acids, ammonia-N, TCA precipitable-N were not affected by the addition of up to 10 ppm supplementary Ni in the diet (Table 5). The propionate level increased ($P<0.05$) while that of butyrate decreased in group T_3. The proportion of acetate was not affected by Ni supplementation. The urease activity was the highest ($P<0.05$) in group T_3 compared to other groups (Spears et al. 1979). Starnes et al. (1982) showed that supplementation of Ni at 5 ppm increased rumen bacterial urease activity regardless of protein source. Fishbein et al. (1976) reported that urease has been found to contain 6 to 8 atoms of Ni per mole of enzyme and is a Ni metalloenzyme. Milne et al. (1990) reported that a diet containing high-energy and low protein with supplementation of Ni at a level of 5 mg/d given in the form of $NiCl_2.6H_2O$ by continuous infusion into the rumen resulted in a significant increase in the rumen urease activity in sheep. Singh et al. (2018) reported that urease activity in the rumen increased significantly due to Ni supplementation in the diet of Hariana heifer as compared to non-supplemental groups. In this study, there was a tendency toward an increase in TCA precipitable N in the 10 ppm Ni supplemented group which might be related to increasing in urease activity. The propionate level increased ($P<0.05$) while that of butyrate decreased in group T_3 as compared to groups T_1 and T_2 showing no significant effect on acetate concentration. O'Dell et al. (1970) also reported that a high level of Ni supplementation in bovine increased the molar percentage of propionic acid and reduced the percentage of butyric acid. Vorob'eva et al. (1962) stated that Ni had a vital role in increasing the growth of propionic acid-producing bacteria in the rumen ecosystem. Spears et al. (1978) reported that supplementation of an adequate protein with Ni in lambs and steers had no significant effect on individual fatty acids (IVFAs_ while a low protein diet with 5 ppm Ni offered to steer significantly increased molar percentage of propionic acid and decreased molar percentage of acetic acid in the rumen liquor compared to control group.

Conclusions

The dietary Ni supplementation at levels of 5 and 10 ppm to the basal diet containing 1.67 ppm Ni did not affect feed intake, growth rate, digestibility of nutrients, N balance, plane of nutrition, pH, total volatile fatty acids, ammonia-N, TCA precipitable-N and acetate in male Murrah buffalo calves. The urease activity and propionate level in rumen liquor increased in group supplemented

Table 4 Effect of supplementation of Ni on the plane of nutrition during metabolism trial

Parameter	Group			SEM	*p*-value
	T_1	T_2	T_3		
BW (kg)	191.15	188.63	192.58	9.28	0.83
DMI (kg/d)	5.18	5.23	5.42	0.12	0.74
DMI (kg/100 kg BW)	2.71	2.77	2.82	0.06	0.83
CP intake (g/d)	654.59	629.47	668.06	8.36	0.16
CP intake (g/100 kg BW)	342.45	333.17	346.90	3.96	0.10
EE intake (g/d)	205.14	188.46	207.64	4.12	0.11
EE intake (g/100 kg BW)	107.32	99.01	107.84	1.48	0.61
NDF intake (kg/d)	2.06	2.08	2.22	0.07	0.66
NDF intake (kg/100 kg BW)	1.08	1.10	1.15	0.31	0.78
ADF intake (kg/d)	1.58	1.56	1.64	0.05	0.85
ADF intake (kg/100 kg BW)	0.83	0.83	0.85	0.02	0.93
OM intake (kg/d)	4.64	4.67	4.84	0.11	0.76
OM intake (kg/100 kg BW)	2.43	2.47	2.51	0.05	0.85
TDN intake (kg/d)	3.38	3.42	3.59	0.08	0.58
TDN intake (kg/100 kg BW)	1.77	1.81	1.86	0.04	0.68
Water intake (L/d)	21.25	21.66	21.75	0.56	0.94
Water intake (L/ kg DMI)	4.10	4.14	4.01	0.10	0.90
Nutritive value (%)					
CP	12.71	12.04	12.34	0.18	0.36
DCP	8.43	8.03	8.24	0.16	0.64
TDN	65.22	65.36	66.22	0.63	0.81

Table 5 Effect of Ni supplementation on rumen fermentation parameters

Parameter	Day	Group			SEM	*p*-value
		T_1	T_2	T_3		
pH	0	6.53	6.43	6.55	0.04	0.59
	60	6.60	6.75	6.63	0.03	0.23
	120	6.70	6.68	6.70	0.31	0.94
	Mean	6.61	6.62	6.63	0.02	0.95
TVFA (mmol /dL)	0	8.49	8.68	8.49	0.20	0.92
	60	10.25	10.18	9.93	0.27	0.89
	120	10.09	10.33	10.03	0.24	0.88
	Mean	9.61	9.73	9.48	0.18	0.80
Acetate (%)	0	66.00	64.64	65.34	1.20	0.92
	60	65.32	66.39	65.24	0.38	0.52
	120	65.06	65.23	65.77	0.42	0.79
	Mean	65.46	65.42	65.45	0.41	0.99
Propionate (%)	0	18.21	18.85	18.26	0.27	0.65
	60	18.92	17.52	22.65	0.80	0.01
	120	18.03	19.95	21.08	0.42	0.01
	Mean**	18.39^{a}	18.77^{a}	20.66^{b}	0.33	0.01
Butyrate (%)	0	15.80	16.51	16.40	0.29	0.66
	60	15.76	16.09	12.11	0.61	0.01
	120	16.91	14.82	13.15	0.64	0.02
	Mean**	16.16^{b}	15.81^{b}	13.89^{a}	0.33	0.01
Ammonia-N (mg/ dL)	0	12.10	12.29	13.42	0.84	0.83
	60	11.73	13.13	14.11	0.84	0.55
	120	12.55	12.92	13.46	0.45	0.61
	Mean	12.13	12.78	13.66	0.50	0.73
TCA precipitable-N (mg/ dL)	0	55.00	54.12	54.30	0.86	0.92
	60	63.84	65.01	66.80	1.79	0.82
	120	64.49	66.65	68.02	1.02	0.10
	Mean	61.11	61.93	63.04	1.11	0.40
Urease activity (µmol NH_3-N /min/mL)	0	2.67	2.59	2.63	0.17	0.98
	60	2.52	3.16	3.45	0.18	0.42
	120	2.72^{a}	3.34^{b}	3.72^{b}	0.15	0.01
	Mean*	2.64^{a}	3.03^{ab}	3.27^{b}	0.11	0.04

[a,b] Values bearing different superscripts in a row differ significantly (*P<0.05; **P<0.01)

with 10 ppm Ni while proportion of butyrate decreased in group T_3 as compared to other groups.

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RESEARCH ARTICLE

Nutritional elucidation of rice- and maize gluten meal-based diets: *in vitro* gas production, digestibility, methane and rumen fermentation

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Abstract: This experiment intends to elucidate the nutritional potential of alternative ingredient – rice gluten meal (RGM) with groundnut cake (GNC) and maize gluten meal (MGM) at incremental levels in the complete diets under *in vitro* system. Nine complete diets were formulated using concentrate mixture, green maize and wheat straw in the ratio of 40:40:20 on a dry basis. The dietary treatments differed in the source of major protein sources used in three concentrate mixtures, i.e., GNC in control, while incremental levels of RGM and MGM – both at 25, 50, 75 and 100% substituted GNC on an isonitrogenous basis. These diets were characterised for chemical composition, protein fractions, *in vitro* gas production profile as well as rumen fermentation attributes. The chemical composition of diets showed a mean crude protein (CP) and total digestible nutrients values of 12.9 and 63.6%, respectively. The range of values obtained for various CNCPS protein fractions (% CP) was 11.3-12.4, 9.42-14.0, 40.9-44.4, 19.1-20.1 and 13.6-14.9 for P_A, P_{B1}, P_{B2}, P_{B3} and P_C, respectively across the diets. Furthermore, rumen undegradable protein was maximum at 100% MGM inclusion and least for the control. However, intestinal protein digestibility did not differ due to treatments. While the GV_{24} was maximum (P<0.01) in the control, it was lowest at 100% MGM. Additionally, *in vitro* dry matter and organic matter digestibilities were higher (P<0.01) for 100% MGM diet and lowest (P<0.01) at 100% RGM level. Moreover, the control diet generated maximum (P<0.01) CH_4 but was minimum (P<0.01) for 100% MGM diet. In addition, values of PF, MBP and MBP:GV_{24} were highest (P<0.01) for 100% MGM and lowest (P<0.01) for control diet. The pH of rumen fluid did not differ; however, concentrations of ammonia nitrogen and total volatile fatty acids were recorded to be maximum (P<0.01) in control and minimum (P<0.01) at 100% MGM. Further, the molar concentration of acetic acid was higher (P<0.01) with MGM-based diets than that of control and RGM diets. Lastly, the proportions of both propionic and butyric acids were minimum with MGM-based diets than with either control or RGM-based diets. Therefore, it could be recommended that RGM could substitute 50-75% of GNC, corresponding to 5-8% inclusion in the complete diet of ruminants. In addition, MGM-based diets excelled in terms of greater digestibility, reduced CH_4 emission as well as beneficially influenced rumen fermentation.

Keywords: Alternative ingredients, *In vitro* fermentation, Maize gluten meal, Protein fractions, Rice gluten meal, Rumen

Introduction

The world is facing the formidable challenge of feeding an ever-increasing human populace, which is projected to reach 9.7 billion by 2070, with the finite available resources (Adam, 2021). Simultaneously, the developing countries are poised to have a greater exigency for livestock-source foods including dairy (OECD/FAO 2021). Under Indian scenario, there is an existing disequilibrium between demand and the availability of feed resources for dairy production. On top of that, driven by commodity inflation in the recent past, most of the traditional ingredients are becoming expensive at farms. Hence, to circumvent the dairy sector incurring economic loss due to high input (feed) costs, there has been a burgeoning research impetus on alternative feed ingredients in ration formulations (Manpreet et al. 2022; Singh et al. 2022). Moreover, alternative ingredients with poor human-edible value may further contribute to sustainable animal diets (Singh et al. 2022). In addition, dietary strategies addressing the reduction of enteric methane (CH_4, a potent greenhouse gas) would be desirable from the viewpoint of cleaner ruminant production since about 40% of global agricultural CH_4

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emission is directly linked with the livestock sector (Pal et al. 2015).

With the increased wet milling of rice, rice gluten meal (RGM) has become available in India as a by-product thereof. Due to its rich crude protein (CP) of approximately 45%, it was investigated recently as the protein alternative for conventional oilseed meals in cattle (Kumar et al. 2016; Malik et al. 2017), buffalo (Mahesh and Thakur 2018) as well as monogastrics (Wani et al. 2021). Whereas, maize gluten meal (MGM) is a similar co-product of corn wet milling with CP value of >60% has been used conventionally in animal feeding (Heuzé et al. 2018). In our previous *in vitro* experiment, it was observed that concentrate mixtures containing 50-75% substitution of groundnut cake (GNC) by RGM and MGM could be possible without affecting digestibility and rumen fermentation. Recently, Manpreet et al. (2022) concluded from an *in vitro* study that soya bean meal (SBM) protein could be completely (up to 100%) replaced by RGM in the concentrate mixture for ruminants. Nonetheless, it becomes imperative to know the effect of inclusion of any dietary treatment in the complete diet of ruminants to gauge its practical utility, as ruminant diets are typically a combination of forages and concentrates at farm conditions. Therefore, in the present experiment, our objective was to evaluate the incremental inclusions of RGM and MGM, isonitrogenously-substituting GNC in the diet on protein fractions, gas production and associated parameters, methane (CH_4), digestibility and rumen fermentation attributes under *in vitro* conditions.

Materials and Methods

Dietary treatments

Nine complete diets were formulated using concentrate mixture, green maize (*Zea mays*) and wheat (*Triticum aestivum*) straw in the ratio of 40:40:20 on a dry basis. The concentrate mixtures were prepared in mash form, while chopped green and dry forages were oven-dried and ground prior to constituting test diets in the laboratory. The dietary treatments differed in the source of major protein ingredients used in three types of concentrate mixtures, i.e., GNC in control, while incremental levels of RGM and MGM – both at 25, 50, 75 and 100% substituted GNC on an isonitrogenous basis. On a physical basis, the range of inclusion of RGM and MGM across various diets was 2.8-11% and 2-8%, respectively (Table 1).

Compositional characterisation of diets

All the diets were analysed for chemical composition like dry matter (DM), organic matter (OM), crude protein (CP; Kjeldahl N × 6.25) and ether extract (EE) as per standard procedures of AOAC (2005). Cell wall fibre fractions like neutral detergent fibre (NDF), acid detergent fibre (ADF) as well as 72% (w/w) H_2SO_4 soluble lignin were estimated by the methods outlined by Van Soest et al. (1991). Heat-stable á-amylase (A-3306, Sigma-Aldrich, USA) was used for NDF estimation, and the values of both NDF and ADF were expressed inclusive of residual ash.

Feed protein fractionation (P_A, PB_1, B_2, B_3 and C) was performed in accordance with the Cornell Net Carbohydrate and Protein System as delineated by Sniffen et al. (1992) (Table 2). Furthermore, feed protein resistant to commercial broad-spectrum protease of *Streptomyces griseus* (type XIV, Sigma P-5147, St. Louis, MO, USA) was regarded as rumen undegraded protein (RUP; Krishnamoorthy et al. 1983), and subsequent to its pepsin-pancreatin digestion, intestinal protein digestion (IPD) was assayed (Calsamiglia and Stern, 1995).

In vitro Hohenheim gas test

A 24 h *in vitro* gas production profile (GV_{24}) of various treatment diets was carried out as per Menke and Steingass (1988). About 200 mg of air-equilibrated substrates of various diets were taken in the glass syringes in triplicate and mixed with the buffered rumen liquor of buffalo steers. These donor animals were fed on maintenance ration with seasonally available forages and concentrate mixture. The DM and OM digestibilities were determined in accordance with Goering and Van Soest (1970) by treating the whole syringe contents with neutral detergent solution (Van Soest et al. 1991). Other parameters such as gas chromatographic measurement of CH_4, partitioning factor (PF; Blümmel et al. 1997), microbial biomass production (MBP) as well as MBP:GV_{24} were calculated as detailed in our previous experiment (Mahesh et al. 2021). Furthermore, rumen fermentation variables, namely pH, ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA) were determined by the standard procedures as described in Mahesh and Mohini (2015).

Statistical analysis

The data generated in the experiment were presented as means along with a pooled standard error of means. Statistical analysis was performed using one-way ANOVA utilising software package of SAS (2012).

Results and Discussion

Chemical composition and protein fractions

The control diet had 12% GNC, whilst the levels of RGM and MGM across diets were in the range of 2.8-11% and 2-8%, respectively (Table 1). Since all the diets were isonitrogenous and MGM had a greater CP (65.2%; Mahesh e al. 2017), its inclusion was relatively less compared with GNC and RGM diets. The chemical composition of diets showed a mean CP and calculated total digestible nutrients values of 12.9 and 63.6%, respectively (Table 3). This nutrient profile is adequate to support the milk production of cows yielding ~ 12-15 kg/d (ICAR, 2013) enabling the results to be applicable for *in vivo* conditions. The

range of values obtained for various CNCPS protein fractions (% CP) was 11.3-12.4, 9.42-14.0, 40.9-44.4, 19.1-20.1 and 13.6-14.9 for P_A, P_{B1}, P_{B2}, P_{B3} and P_C, respectively across diets. It is apparent that since the protein of GNC is highly rumen degradable in nature (NASEM 2021), the corresponding value of P_A was greatest in the control. By contrast, fractions P_{B2} and P_{B3} were greater in diets containing RGM and MGM, which are reported to be less rumen degradable (Mahesh et al. 2017; Mahesh and Thakur 2018). Furthermore, this has been justified by the actual RUP values obtained in the present study, showing maximum ($P<0.01$) at 100% MGM inclusion and least ($P<0.01$) for the control. However, IPD did not differ due to treatments; the value averaged 75.0%. The three major test protein feeds employed in the study, i.e., GNC, RGM and MGM contained 43.1, 47.5 and 65.2% CP, respectively (Mahesh et al. 2017). It could be deduced that the variation in the compositional parameters such as EE, fibre fractions, CHO and NFC was a function of inherent characteristics of the test

Table 1 Ingredient composition of complete diets (% DM) used in the experiment

Component	Control	Level of RGM (%)				Level of MGM (%)			
		25	50	75	100	25	50	75	100
Forage									
Green maize	40	40	40	40	40	40	40	40	40
Wheat straw	20	20	20	20	20	20	20	20	20
Concentrate mixture									
Maize	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
Groundnut cake	12	8.4	6.0	3.2	-	9.0	6.0	3.6	-
Rice gluten meal	-	2.8	5.5	8.2	11	-	-	-	-
Maize gluten meal	-	-	-	-	-	2.0	4.0	6.0	8.0
De-oiled rice bran	7.2	8.0	7.6	7.6	8.0	8.0	9.0	9.4	11
Wheat bran	6.4	6.4	6.5	6.6	6.6	6.6	6.6	6.6	6.6
Mineral mixture[a]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Salt (as NaCl)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4

[a]Bureau of Indian Standards-compliant type-II mixture comprising of macrominerals, trace minerals and vitamins

DM: dry matter; RGM: rice gluten meal; MGM: maize gluten meal

Table 2 CNCPS protein fractionation scheme employed for diet characterization

Fraction	Constituent	Degradation pattern	Calculation
P_A	NPN compounds: ammonia, amino acids, nitrates and peptides	Instantaneously degradable in the rumen and none reaches intestine	P_A = NPN (% SP) × 0.01 × SP (% CP)
P_{B1}	Globulins and some albumins	Rapidly degradable in the rumen and completely digestible at intestine	P_{B1} = SP (% CP) – P_A (% CP)
P_{B2}	Most albumins and glutelins	Intermediately degradable in the rumen and completely digestible at intestine	P_{B2} = 100 – P_A (% CP) – P_{B1} (% CP) – P_{B3} (% CP) – P_C (% CP)
P_{B3}	Prolamins, cell wall (extension) proteins and denatured proteins	Slowly degradable in the rumen	P_{B3} = NDICP (% CP) – ADICP (% CP)
P_C	Maillard products, N bound to lignin/tannins	Undegradable in the rumen and unavailable at intestine	P_C = ADICP (% CP)

Source: Sniffen et al. (1992) and Licitra et al. (1996)

CNCPS: Cornell net carbohydrate and protein system; CP: crude protein (N×6.25); NPN: non-protein nitrogen, determined as the difference between total crude protein and trichloroactetic acid-precipitable crude protein; SP: borate-phosphate buffer soluble protein; NDICP: neutral detergent-insoluble crude protein; ADICP: acid detergent-insoluble crude protein

Table 3 Chemical composition and protein fractions of various dietary treatments

	Control	Level of RGM (%)				Level of MGM (%)				SEM
		25	50	75	100	25	50	75	100	
Chemical composition (% DM)										
CP	12.9	12.8	12.9	12.9	12.9	12.9	12.9	12.9	12.9	-
EE	3.07	2.89	2.78	2.65	2.49	2.96	2.84	2.76	2.61	-
Ash	8.22	8.27	8.24	8.23	8.26	8.21	8.21	8.17	8.21	-
NDF	51.2	51.7	52.1	52.5	53.0	51.1	51.0	50.9	50.8	-
ADF	33.1	33.1	33.2	33.2	33.2	32.9	32.8	32.7	32.5	-
Hemicellulose[a]	18.1	18.6	18.9	19.3	19.8	18.2	18.2	18.2	18.3	-
Cellulose[b]	27.2	27.2	27.4	27.4	27.5	27.1	27.1	27.1	27.0	-
ADL	5.95	5.88	5.82	5.76	5.70	5.85	5.75	5.65	5.54	-
CHO[c]	74.8	75.1	75.0	75.0	75.2	74.8	74.7	74.5	74.6	-
NFC[d]	23.6	23.4	22.9	22.5	22.2	23.7	23.7	23.6	23.8	-
TDN[e]	63.5	63.5	63.4	63.4	63.4	63.7	63.8	63.9	64.1	-
Protein fraction[f] (% CP)										
P_A	12.4	12.3	12.3	12.3	12.2	12.1	11.8	11.6	11.3	-
P_{B1}	14.0	12.5	11.4	10.2	8.84	12.8	11.7	10.7	9.42	-
P_{B2}	40.9	41.6	42.3	42.9	43.7	41.8	42.7	43.5	44.4	-
P_{B3}	19.1	19.3	19.2	19.2	19.4	19.3	19.6	19.7	20.1	-
P_C or ADICP	13.6	14.3	14.7	15.4	15.9	13.9	14.2	14.5	14.9	-
RUP (% CP)	36.4^{d}	37.8^{cd}	38.9^{bc}	40.1^{ab}	41.5^{a}	37.7^{cd}	39.1^{bc}	40.2^{ab}	41.8^{a}	0.60
IPD (% RUP)	75.4	75.2	75.1	75.0	74.8	75.2	75.0	74.9	74.6	0.41

[a]NDF–ADF; [b]ADF–ADL; [c]100–CP–EE–ash; [d]CHO–NDF; [e]92–0.86×ADF (Owens et al. 2010); [f]Expressed as rounded percentage, and hence cumulative fractions (P_A to P_C) may not add exactly to 100

Means bearing different superscripts in a row differ significantly (P< 0.01)

RGM: rice gluten meal; MGM: maize gluten meal; DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; CHO: carbohydrates; NFC: non-fibrous carbohydrates; TDN: total digestible nutrients; RUP: rumen undegradable protein; IPD: intestinal protein digestibility

Table 4 *In vitro* gas production and associated parameters of various dietary treatments

Treatment	GV_{24} (mL/g)	IVDMD (%)	IVOMD (%)	CH_4 (mL/g)	PF	MBP (mg)	MBP: GV_{24}
Control	178^{a}	65.6^{cd}	67.0^{c}	52.8^{a}	3.77^{e}	279^{d}	1.57^{e}
Level of RGM (%)							
25	172^{b}	65.4^{cd}	67.0^{c}	52.4^{a}	3.90^{cde}	293^{cd}	1.70^{cde}
50	169^{bc}	64.5^{d}	66.1^{c}	51.3^{ab}	3.90^{de}	289^{d}	1.70^{de}
75	163^{def}	63.2^{e}	64.8^{d}	49.3^{bc}	3.98^{cd}	289^{cd}	1.78^{cd}
100	159^{ef}	62.5^{e}	64.0^{d}	48.3^{c}	4.03^{cd}	290^{cd}	1.83^{cd}
Level of MGM (%)							
25	166^{cd}	66.0^{bc}	67.3^{bc}	51.1^{ab}	4.05^{c}	307^{c}	1.85^{c}
50	163de	66.9^{ab}	68.6^{ab}	50.5^{ab}	4.20^{b}	327^{b}	2.00^{b}
75	159^{f}	67.1^{ab}	68.9^{a}	49.6^{ab}	4.34^{b}	339^{b}	2.14^{b}
100	150^{g}	67.9^{a}	69.4^{a}	48.2^{c}	4.63^{a}	364^{a}	2.43^{a}
SEM	1.28	0.37	0.38	0.67	0.04	5.19	0.04

Means bearing different superscripts in a column differ significantly (P<0.01)

RGM: rice gluten meal; MGM: maize gluten meal; GV_{24}: gas produced after 24 h of incubation

IVDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility; CH_4: methane; PF: partitioning factor; MBP: microbial biomass production

ingredients since the forage level remained constant. Such a trend has been noted previously for concentrate mixtures prepared with the incremental levels of these ingredients (Mahesh et al. 2021).

In vitro gas production and associated parameters

While the GV_{24} was maximum (P<0.01) in the control diet, it was gradually decreased (P<0.01) as the level of RGM and MGM increased, and found lowest at 100% MGM (Table 4). *In vitro* digestibilities of DM and OM were higher (P<0.01) for 100% MGM diet and lowest (P<0.01) at 100% RGM level. Furthermore, the control diet produced the most (P<0.01) CH_4, whereas the 100% MGM diet produced the least (P<0.01). In addition, values of PF, MBP and MBP:GV_{24} were highest (P<0.01) for 100% MGM and lowest (P<0.01) for control diet (Table 4).

The gas production in the rumen is a reflection of the fermentability of feed substrates. A relatively greater GV_{24} obtained for GNC-based control depicts its rapid rate of fermentation compared with RGM- and MGM-based diets. This is in general agreement with the previous reports for RGM (Kumar et al. 2016) and MGM (Lamba et al. 2016). Although GV_{24} reflects OM digestion and thus energetic feed value (Menke and Steingass 1988; Pal et al. 2015), diets containing MGM showed maximum digestibility despite producing low GV_{24}, as has already been reported by earlier researchers (Lamba et al. 2016; Prusty et al. 2017; Mahesh et al. 2021).

Ruminal production of CH_4 is a result of the fermentative digestion of complex feed nutrients. Previously, when a range of protein ingredients were evaluated, it was evident that MGM produced low GV_{24} as well as CH_4 (Lamba et al. 2016; Prusty et al. 2017) as seen with MGM-based diets in the present study. Additionally, our data on RGM is consistent with that of Manpreet et al. (2022), who noticed a lower *in vitro* CH_4 formation when RGM was substituted for 100% of SBM in the concentrate mixture.

Being an index of the efficiency of microbial protein synthesis, the values for PF across various diets fall closely within the theoretical range of 2.74-4.41 (Blümmel et al. 1997). Moreover, it is reasonable that owing to a greater digestibility per unit of GV_{24}, diets based on MGM yielded greater values for MBP:GV_{24}.

In general, the different *in vitro* variables tested in the current experiment are in line with previous studies that used a 60:40 ratio of forage:concentrate as substrates and evaluated using an *in vitro* gas technique (Getachew et al. 2004; Patra et al. 2006; Goswami et al. 2012; Kumar et al. 2013).

Rumen fermentation characteristics

There was no difference in the pH of the clarified rumen fluid across various diets (Table 5). However, concentrations of NH_3-N and total VFA were recorded to be maximum (P<0.01) in control and minimum (P<0.01) at 100% MGM. The fractionation of VFA revealed that the molar concentration of acetic acid was higher (P<0.01) with MGM-based diets than that of control and RGM diets. The proportions of both propionic and butyric acids were minimum with MGM-based diets than with either control or RGM-based diets (Table 5).

The pH is critical for optimum fibre digestion by rumen microbial consortia and all the nine diets recorded pH within the physiological range of 6.7-6.9. A drastic deviation of pH was not expected since the diets evaluated contained 60% of forage that is sufficient to maintain buffering, and the treatments differed only in the type of protein sources rather than energy. It appears that protein degradation in the rumen has influenced NH_3-N, whose concentration was lower with diets having relatively high

Table 5 *In vitro* rumen fermentation characteristics of various dietary treatments

Treatment	pH	NH_3-N (mg/dL)	Volatile fatty acid (mol/100 mol)		
		Total	Acetic acid	Propionic acid	Butyric acid
Control	6.53	17.0^{a}	94.4^{a}	70.3^{b}	19.4^{c}	10.3^{a}
Level of RGM (%)						
25	6.56	16.3^{bc}	93.2^{ab}	69.8^{bc}	19.9^{bc}	10.3^{a}
50	6.53	15.5^{d}	92.7^{ab}	69.3^{bc}	20.4^{ab}	10.3^{a}
75	6.60	14.8^{f}	92.3^{ab}	69.3^{bc}	20.6^{ab}	10.2^{a}
100	6.56	14.1^{g}	91.4^{b}	68.8^{c}	21.1^{a}	10.1^{a}
Level of MGM (%)						
25	6.53	16.5^{b}	85.7^{c}	71.3^{a}	20.0^{bc}	8.67^{b}
50	6.50	15.8^{cd}	83.5^{d}	71.6^{a}	19.7^{bc}	8.74^{b}
75	6.53	15.4^{de}	81.1^{e}	72.1^{a}	19.5^{c}	8.49^{b}
100	6.56	14.9^{ef}	79.1^{e}	72.2^{a}	19.4^{c}	8.43^{b}
SEM	0.04	0.13	0.58	0.30	0.26	0.18

Means bearing different superscripts in a column differ significantly (P<0.01)
RGM: rice gluten meal; MGM: maize gluten meal; NH_3-N: ammonia nitrogen

RUP (i.e., 100% MGM-diets). A greater concentration of total VFA in the control diet could be explained by the fact that GV_{24} and VFA production are highly positively correlated (Getachew et al. 2004; Singh et al. 2010). Indeed, the pattern of individual VFA produced in rumen could be closely related to the type of substrate being fermented. It is well established that lower propionic and butyric acids are associated with a low GV_{24} (Singh et al. 2010), which reaffirms the minimum GV_{24} exhibited by the MGM-based diets.

The overall results of the experiment infer that most of the studied parameters corroborated with the earlier report on concentrate mixtures formulated with graded levels (0, 25, 50, 75 and 100%) of RGM and MGM (Mahesh et al. 2021). As a result, forage components of diets, such as green maize and wheat straw, appeared to have a minimal associative effect on the studied response variables. However, this warrants *in vivo* substantiation through feeding trials.

Conclusions

The *in vitro* evaluation of diets containing incremental levels of gluten meals in this study revealed that RGM could be recommended at 50-75% substitution of GNC, corresponding to 5-8% inclusion in the complete diet of ruminants. In addition, MGM-based diets excelled in terms of greater digestibility, reduced CH_4 emission as well as beneficially influencing rumen fermentation.

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RESEARCH ARTICLE

Effect of purified waste water intake on physiological, growth and health parameters in crossbred (Alpine x Beetal) lactating goats

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Abstract: Present study in goat (*Capra hircus*) was conducted to examine the effect of purified waste water on the physiological responses, health status and growth parameters in comparison to the control group goats maintained on fresh water. The study was carried out in lactating goats maintained at Livestock Research Centre, ICAR-NDRI, Karnal during November to January 2018-19. The dry matter and water intake of the lactating goats non-significantly ($p<0.05$) decreased in the treatment groups as compared to the control group goats provided with fresh water. The different physiological responses (Heart Rate, Respiration Rate and Rectal Temperature) and hematological parameters (TEC, TLC, Hb, PCV, ESR, and DLC) of the goats provided with purified waste water although differed from the control values but the differences were non-significant ($p<0.05$). The growth parameter which included body weight, remained non-significantly ($p<0.05$) different in treatment groups as compared to the control groups. Milk yield in the crossbred lactating goats differed non-significantly ($p<0.05$) in the treatment group as compared to the control group. The parasitological infestation was found to be positive for *Moneizia expansa* and *Giardia sp.* some of the faecal samples of both the groups i.e. control as well as treatment groups. Hence, the purified waste water may be recommended as drinking water to the crossbred lactating goats in the water scarce areas.

Keywords: Goat, Growth, Physiological responses, Water scarcity, Waste water treatment

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Introduction

Livestock are integrated part of agricultural production system of India. Goats are particularly known to be a useful resource for poor people living in the arid and semi-arid regions as they can sustain on scanty and dry forages under extreme climatic conditions meanwhile, other species of livestock often perish in these regions. Majority of the sheep and goat producers in India are from the arid and semi-arid regions, which are characterized by frequent droughts and fluctuating precipitations and the animals reared in these regions are known to face water scarcity and low nutritive feeds (Faroda, 1999). At present, due to climate change water has become a very limiting resource affecting the sustainability of livestock and agriculture (Malley et al. 2009; Tarawali et al. 2011). Therefore, in future days the water availability will become more limited due to the intensified effects of climate change and unpredictable weather resulting in irregular rainfalls (Jaber et al. 2013).

The livestock especially the small ruminants that are adapted to the arid regions have the ability to select high quality forages from the scanty vegetation prevailing in these regions and thus able to maintain a relatively similar basal diet quality during different seasons. These arid adapted animals otherwise reduce their feed intake significantly in extreme dry seasons as the quality and quantity of forage biomass. According to (Alamer, 2006) small ruminants, particularly goats, may stay in a dehydration state for several days when in the summer season they experience water scarcity Adaptability of drought tolerance enables races of sheep and goats to graze far away from watering points even 50 km or further away (Bayer and Feldmann, 2003) for several days and resourcefully exploit irregular desert pastures (Alamer, 2006).Therefore, selection of these drought tolerant animal breeds is very valuable for sustaining the animal production under a progressively challenging environment of water scarcity (Iniguez, 2005).

Limiting water availability to livestock will depress production rapidly and severely, and poor-quality drinking water is often a factor limiting intake. Therefore, the present study was done to investigate if goats may sustain on treated waste water which

fulfills all the drinking standards without being physiologically affected.

Materials and Methods

Geographical location of the study area

The experiments were conducted on the crossbred (Alpine X Beetal) lactating goat present at ICAR-NDRI, Karnal which is situated in Haryana state of India at an altitude of 240 meters above the mean sea level and at 29°42'3" N latitude and 76°59'6" E longitude. The maximum temperature recorded goes beyond 45°C in summers and minimum temperatures drops to 2°C in winters. The average rainfall is about 766 mm.

Experimental animals

Goats used in the study were crossbred (Alpine X Beetal) lactating goats. The average weight of lactating goats was 46.58 kg and those animals which were free from any reproductive, anatomical and physiological disorders were selected. Total twelve lactating female goats (1.5-2 years old), were selected from the Livestock Research Centre, ICAR-NDRI, Karnal. The experimental animals were maintained under proper housing system, provided with wind break curtains and paddy straw concrete flooring, hence the temperature maintained near to their comfort zone. All the studies are carried out under farm conditions of ICAR-NDRI, Livestock Research Centre, from November to January under prevailing management conditions. These lactating goats were further divided equally into two groups, six animals in each group, i.e. control and treatment. The feeding and management practices for both groups were followed as per the feeding standards of Livestock Research Centre, ICAR-NDRI.

Waste water purification for drinking

The waste water from institute's Effluent Treatment Plant (ETP) was collected in 1000 liters capacity container and 1000 grams (1 Kg) powdered aluminium sulfate (industrial alum) was mixed thoroughly and kept for 2 hours for complete coagulation and settlement. After this coagulation, 500 grams powdered activated charcoal (PAC)/ dung cake fresh ash was added and mixed thoroughly. This mixed water was allowed to stand 8-10 hours in the same container and clear water was decanted in another container with simple muslin cloth filter and 250 ml 1% sodium hypochlorite solution was thoroughly mixed in water before use to make it free from biological infections. The obtained purified water was analyzed for different quality parameters for use in livestock drinking purpose. All the parameters were within the range of different national and international drinking water standards like IS: 10500, 2012; APHA and EPA standards). The underground tube well water available at Livestock Research Center (LRC), ICAR-NDRI, was used for control group animals. The underground water was also analyzed for its physical, chemical and microbiological parameters .

Analysis of water parameters

Water temperature, turbidity, total dissolved solids, alkalinity, electrical conductivity, oxidative reduction potential, pH and dissolved oxygen were studied by microprocessor soil and water analysis kit.

Water microbial parameters

Total coliform count was done by the Most Probable Number (MPN) method and total bacterial count was done by Total Viable Count method (TVC).

Water intake

Water was offered twice a day at 9 AM and 3 PM. Quantified water was offered ad libitum to the animals and the leftover water was measured to determine the water intake by calculating the difference between the initial amount of water and leftover amount of water.

Physiological responses of lactating cross bred goats

Rectal temperature (°F) was recorded with a digital thermometer by keeping the thermometer in contact with rectal mucosa for 2 min. Respiration rate was measured by observing the inward and outward abdominal movements in one minute. One inward and outward abdominal movement was counted as one respiration and results were expressed as breaths per min. Heart rate per minute was recorded by placing the stethoscope between the left third and fifth intercostal space and the results are expressed were expressed as beats per min.

Blood sample collection and analysis

Blood samples were collected from the jugular vein of the goats in EDTA vacutainer tubes and then immediately placed in the icepacks and brought to the laboratory. Hemoglobin (%) was measured by using Sahli's hemoglobinometer, TEC was done by using Neubauer's (Hemocytometer) method, PCV (%) was measured by micro-hematocrit method, Total leucocyte count ($x10^3$/ml) as well as DLC (%) were analyzed by the Neubauer's counting chamber and ESR (mm/12hrs.) was studied by Westergren method.

Dry matter (DM) of feed

A measured quantity of feed sample in a dry aluminium tray was taken and placed in hot air oven to dry at 100 ± 5°C for 24 hours. The loss in moisture content after drying was estimated and the dry matter was calculated as follows:

$$DM(\%) \frac{(\text{Wt. of Aluminium tray + sample after drying}) - \text{Wt. of Aluminium tray}}{\text{Wt. of fresh sample taken in Aluminium tray}} \times 100$$

Wt. of fresh sample taken in Aluminium tray

Dry matter intake (DMI)

The dry matter intake (kg/day/animal) of experimental animals were recorded on consecutive two days in a fortnight throughout experimental period, by subtracting residual dry matter from the quantity of dry matter offered.

Body weight

Body weight (kg) was measured by using weighing balance during morning hours.

Milk yield and parasitological examination

Milk yield was recorded at morning and evening in kg/animal/day. Faecal sample were collected at fortnight intervals and examined by the direct faecal examination and cross checked by the concentration method.

Statistical analysis

Statistical analysis of the obtained data was performed using software version (22) of the SPSS system. Statistical analysis of the data was carried out to find mean ± S.E. Independent T-test and paired T-test were done to find out the significant difference between treatments and fortnight intervals. The pair wise comparison of means was carried out using post-hoc Duncan multiple comparison tests.

Results and Discussion

The present experiment was conducted on goat (*Capra hircus*) to study the effect of purified waste water intake on their physiological status, hematological, health and body weight parameters. The results related to change in physiological status, health and growth parameters of crossbred lactating animals have been presented below.

Dry matter and water intake of cross lactating goats maintained on fresh and purified waste water

The samples were taken at fortnight intervals and the values of dry matter and water intake have been presented in (Table 1). In case of control group, the dry matter intake was recorded between 1.55 to 1.71 (kg/animal/day) and water intake ranged between 2.83 to 4.07(liter/animal/day), respectively. Whereas, in the treatment group the dry matter intake and water intake were recorded between 1.49 to 1.60 (kg/animal/day), 2.67 to 3.65 (liter/day/animal), respectively. The values recorded were found to be non-significantly ($p<0.05$) different between the groups.

The water intake in experimental crossbred lactating goats in both control and treatment group was non-significantly different during the experimental period. The water intake (liters/day) was found to be directly related to the dry matter intake (kg/day) by the lactating goats of control and treatment groups. Dry matter intake in crossbred lactating goats showed a decrease in different fortnight. The maximum dry matter intake was reported in first fortnight and lowest were reported in fifth fortnight. There were significant changes in water intake from first to fifth fortnight. The decrease in the dry matter intake may be attributed to characteristics of lactation as the lactation curve advances the milk yield started declining and dry matter required for lactating goats reduced significantly as reported by (Mleil et al. 2011). The water intake and dry matter intake pattern values are in agreement and in range with the

findings of (Ehrlenbruch, 2010 and Francis 2017). The values of milk yield (kg/day) for lactating goats were in agreement with (Mengistu et al. 2007; Thang et al. 2012)

Physiological responses of cross lactating goats maintained on fresh and purified waste water

The mean±SEM values of fortnightly recorded physiological responses in lactating goats presented in (Table 1). The heart rates (beat/min) for control group goats were ranged between 75.67 to 78.33 (beats/min), whereas, in treated group these values ranged between 76.33 to 78.33 (beats/min.) The heart rate (beats/min.) found to be non-significantly ($p<0.05$) different between the two groups. Similarly respiratory rate (breaths/min.) for control group goats ranged between 23.67 to 26.17 and for treatment

Table 1 Mean (±SEM) values of dry matter, water intake, physiological responses, growth parameters in control and treatment groups of cross bred lactating goats

Parameters	Control			Treatment		
Feed intake	Min.	Max	Mean	Min.	Max	Mean
DMI (kg/animal/day)	1.55	1.71	1.61±0.02	1.49	1.60	1.54±0.01
WI (liter/animal/day)	2.83	4.07	3.42±0.10	2.67	3.65	3.16±0.10
Physiological responses						
HR (beat/min)	75.67	78.33	76.60±0.44	76.33	78.33	77.67±0.66
RR (breaths/min.)	23.67	26.17	25.00±0.42	23.83	28.33	25.70±0.60
RT(^{0}F)	100.98	101.67	101.22±0.16	100.77	100.97	101.29±0.16
BW (Kg)	44.42	49.33	46.58±0.64	43.42	46.08	44.68±1.42

Dry matter intake (DMI), Water intake (WI), Pulse rate (PR); Heart rate (HR), Rectal temperature (RT), Body weight (BW); No significant differences were observed between control and treatment groups ($P<0.05$).

group it varied between 23.83 to 28.33 (breaths/min.). The respiratory rate varied non significantly ($p<0.05$) between both the experimental groups. The data on rectal temperature (^{0}F) also varied non-significantly ($p<0.05$) between control and treatment group goats which ranged between 100.98 (^{0}F) to 101.67 (^{0}F) for control group and 100.77 (^{0}F) to 100.97 (^{0}F) in treatment group (Table 1).

In the present study it was found that there were no significant differences in the physiological responses of lactating goats maintained on control (fresh water) and treated (purified waste water) water. The experimental animals were maintained under proper housing system, provided with wind break curtains and paddy straw concrete flooring, hence the temperature maintained near to their comfort zone. The values of physiological responses were within the ranges as reported by (Darcan et al. 2008; Phulia et al. 2010; Mdletshe et al. (2017; Francis, 2017). The treated water was fulfilling all the required standards as per (Dairy NRC, 2001, EPA, 2002) hence no bacterial or parasitic contamination was caused to the experimental animals. The treatment group also remained healthy as that of controlled group in lactating goats. The animals infected with different bacterial infections and ecto/endo parasites may exhibit higher physiological reactions due to different type of biotic stresses as reported by (Akkari et al. 2011; Khayeche et al. 2014; Silva et al. 2015).

Body weight of cross lactating goats maintained on fresh and purified waste water

Mean±SEM values of body weight in crossbred lactating goats maintained on fresh and purified waste water ranged between 44.42 to 49.33 (kg) in control group and 43.42 to 46.08 (kg) in treated group. The difference in body weights of both the groups found to be varied non- significantly ($P<0.05$).

The growth parameters like body weight (kg) of experimental lactating goats of control as well as treated groups showed no

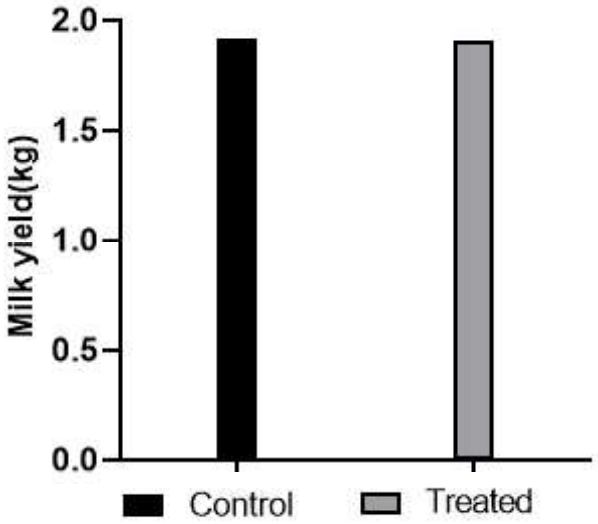

Fig. 1 Milk yield in control and treatment groups of cross bred lactating goats

significant differences. Both the groups were receiving water free from all contaminants and fulfilling all the standards (BIS 1991; Dairy NRC, 2001; EPA, 2002) of drinking water recommended to livestock. The lactating goats receiving purified/ treated water showed a linear pattern in all growth parameters in all five fortnights as that of control group crossbred lactating goats which received fresh water. The growths of crossbred lactating goats of both the experimental groups are in agreement with the findings (Birteeb et al. 2015). The crossbred lactating goats also in both control and treatment group showed normal growth pattern when compared to each other. Similar results were obtained by (Yacout et al. 2015). Hence there was no deleterious effect on the growth parameters of crossbred lactating goats

Heamatological parameters of cross lactating goats maintained on fresh and purified waste water

Table 2 Mean (±SEM) values of hematological parameters in control and treatment groups of cross bred lactating goats

Parameters	Control			Treatment		
	Min.	Max	Mean	Min.	Max	Mean
Hematological parameters						
TEC (million/µl)	16.58	18.81	17.45±0.54	15.63	18.41	17.08±0.48
TLC (thousand/µl)	10.29	13.14	12.08±0.37	11.82	13.45	12.86±0.34
Hemoglobin (%)	7.67	8.00	7.80±0.16	7.41	8.30	7.79±0.13
PCV (%)	28.17	29.97	29.42±0.61	27.95	30.15	29.26±0.48
ESR (mm/12hrs)	0.17	1.00	0.63±0.07	0.33	1.33	0.90±0.08
Differential Leucocyte Count (%)						
Neutrophils,	32.80	36.73	34.96±0.85	33.18	37.25	35.71±0.60
Eosinophils,	1.33	2.33	1.87±0.16	1.83	2.50	2.20±0.13
Lymphocyte	47.72	57.26	52.61±1.33	51.75	55.45	53.89±1.22
Monocyte	1.83	2.33	2.03±0.06	1.67	2.33	1.90±0.06

Total Erythrocyte Count (TEC); Total Leukocyte Count (TLC); Packed cell volume (PCV); Erythrocyte Sedimentation Rate (ESR)

Fig. 2 *Moneizia expansa* egg and *Giardia sp.* cyst found in the faecal sample of crossbred lactating goats

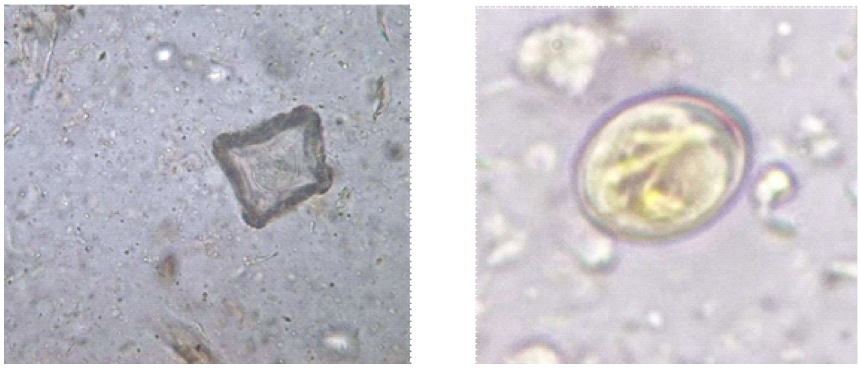

Table 3 Mean (±SEM) values of parasitological status of lactating goats maintained on fresh and purified waste water

Control Sample						Treatment Sample					
1	2	3	4	5		1	2	3	4	5	
Adult/Egg					IR (%)	Adult/Egg					IR (%)
+	-	-	-	-	20	-	-	-	-	-	0
-	+	+	+	+	80	+	+	+	-	+	80
+	-	-	-	-	20	-	-	-	-	-	0
-	-	-	-	-	0	-	-	-	-	-	0
+	-	-	-	-	20	-	+	+	+	-	60
++	+	+	+	+	100	+	-	+	-	+	60

+: presence of *Moneizia expansa* or *Giardiasp.* - : absence of any parasite IR:Infestation Rate

Mean±SEM of hematological values like Total Erythrocyte Count (TEC million/µl)), Total Leucocyte Count (TLC million/µl), Hemoglobin (Hb %), Packed Cell Volume (PCV %), Erythrocyte Sedimentation Rate (ESR mm/12hrs) in control as well as in treatment groups of crossbred lactating goats have been depicted in (Table 2). TEC, TLC, Hb, PCV and ESR, varied non- significantly ($P<0.05$) between both the groups.

The values for DLC (%) in terms of Neutrophils 32.80 to 36.73, Eosinophils1.33 to 2.33, Basophils0.17 to 0.50, Lymphocyte 47.72 to 57.26 and Monocyte (1.83 to 2.33 %) in control group goats. Similarly, the values of these parameters in treatment groups were found to be ranged between 33.18 to 37.25 for neutrophil, 1.83 to 2.50 in eosinophil, 0.17 to 0.50 in basophil, 51.75 to 55.45 in lymphocyte and in monocyte it ranged between 1.67 to 2.33. The group mean for control was 34.96±0.85 in neutrophils, 1.87±0.16 in eosinophils, 0.37±0.09 in basophils 52.61±1.33 in lymphocytes and 2.03±0.06 in monocytes. In treatment group the mean values were found to be 35.71±0.60 in neutrophils, 2.20±0.13 in eosinophils, 0.33±0.10 in basophils 53.89±1.22 in lymphocytes and 1.90±0.06 in monocytes (Table 2).

All the values of DLC parameters between and within both the groups were found to be non-significantly ($p<0.05$) different whereas only the values of lymphocytes were found to be significantly ($p<0.05$) different within the control group.

The haematological parameters like TEC, TLC, Hb, PCV, ESR and DLC of experimental lactating goats of control as well as treated group showed no significant differences. Both the groups were receiving water free from all contaminants and fulfilling all the standards (Dairy NRC, 2001; EPA, 2002) of drinking water recommended to livestock. The haematological parameters of crossbred lactating goats of both the experimental groups are in normal range of as studied by (Upadhyay and Rao 1985; Phulia et al. 2010; Francis et al. 2017). Hence, there was no deleterious effect on the growth parameters of crossbred lactating goats.

Milk yield of cross bred lactating goats maintained on fresh and purified waste water

Mean±SEM values of milk yield of lactating goats in control and treated groups have been depicted in (Fig.1). The Mean±SEM values of milk yield (Kg/day/animal) of lactating goats ranged between 1.75 to 2.07 (kg/day) in control group and 1.82 to 2.13 (kg/day) in treatment group, which were found to be non-significantly ($p<0.05$) different. The group mean for milk yield in control and treated group was 1.92±0.04 (kg/day) and 1.91±0.03 (kg/day) respectively.

Parasitological status of cross bred lactating goats maintained on fresh and purified waste water

The parasitological status of lactating goats has been depicted in (Table 3 and Fig. 2.) For control group five samples of each crossbred lactating goats were collected at fortnight intervals. During the examination of adult/egg (*Moneizia expansa, Giardia sp.*) infestation rate, only six animals were reported infested with

adult/egg of the above-mentioned parasites. Out of six lactating goats from the control and treatment group which received fresh water and purified waste water respectively, three lactating goats were found to be infested with adult/egg (*Moneizia expansa, Giardia sp)*.

The parasitological status of the crossbred lactating goats under control and treatment groups revealed that there were no significant differences in parasitic infestation of *Girordia sp* and *Monezia expansa.* The findings of this study are in agreement with the results observed by (Salem et al. 2011; Khayeche et al. 2014). In the present study, results showed that the purified waste water offered to the treatment groups of crossbred lactating goats was fulfilling all the recommended biological parameters (BIS 1991; Dairy NRC 2001; EPA, 2002). Water recycling experiences prove to be effective and successful in creating a new source of water supply without comprising the livestock health. Recycling waste water and grey water with organic matter is easier than treating the chemical mixed waste water. Water recycling/ purification along with water conservation techniques may help us to sustainably manage our natural resources.

Conclusion

Since the purified waste water has no detrimental effects on productivity of crossbred lactating goats, it may be concluded that the purified waste water offered to lactating goats was fulfilling all the standards of livestock drinking water. Thus, waste water recycling is effective and efficient tool in creating a new source of water supply without comprising animal's welfare and their health status.

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RESEARCH ARTICLE

Economic analysis of milk production in Southern and North coastal regions of Andhra Pradesh

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Abstract: A study on economic analysis of milk production was carried out in Chittoor and Vishakhapatnam districts of Andhra Pradesh. Using the cumulative square root frequency method, farms were divided into small, medium, and large groups based on the number of milch animals. A sample of 80 dairy farmers as drawn randomly from selected four villages of Chittoor and Vishakhapatnam districts of Andhra Pradesh during 2019-20. The cost and return calculation was computed using the budgeting technique. Fixed costs and variable costs were considered for estimation using the capital recovery cost technique. The overall maintenance cost per day per animal was highest for buffalo, followed by crossbred and indigenous cows. The overall cost of milk production was highest in the case of indigenous (₹ 32.45/ litre) cow followed by buffalo (₹ 30.31/litre) and crossbred (₹ 22.64/litre). Study revealed that buffalos were found to be more profitable than crossbred and indigenous cows. Milk productivity was highest for crossbred cows as compared to indigenous cows and buffalos. Because of higher market price of milk for buffalo and comparative lower maintenance cost, overall net returns of buffalo were higher than crossbred and indigenous cows.

Keywords: Milk Production, Capital Recovery Cost, Standard Animal Unit

Introduction

In India, dairying plays a vital role in providing income and employment to masses. Coupled with crop production, dairy farming is practiced in rural households from ancient time. Indian dairy industry witnessed a great transformation from the milk scarcity to the largest global milk producer. With 221.1 million tonnes of milk production and per capita milk availability of 444 g/day, India is world's largest producer and consumer of milk (GOI 2022). Milk production has increased significantly as a result of extensive dairy development programs and increased consumer demand for value-added products. The cost of milk production is an important tool for assessing the economics of dairy businesses at the producer level and determining procurement prices at dairy cooperative society (Sunil, et al. 2016). Andhra Pradesh is primarily an agricultural state with bright milk production potential (Government of Andhra Pradesh, 2017-18).Andhra Pradesh has well-known cattle breeds such as Ongole and Punganur, as well as the Buffalo (Godavari). In 2018-19, it ranked fifth in buffalo population (10.6 million) and fourth in buffalo milk production (7.4 million tonnes). Milk production in Andhra Pradesh increased to 150.44 lakh tonnes (LT) in thelast decade. (NDDB, 2018-19). On average, dairy production (with 1 or 2 dairy animals) adds net monthly household income of ₹ 1070 in Andhra Pradesh, which is roughly one third of the monthly wage of an agricultural wage labourer (Squicciarini and Vandeplas, 2011). In the light of importance of dairy sector for rural upliftment, an attempt has been madeto analyse the economics of milk production in Andhra Pradesh.

Materials and Methods

Selection of study area

Andhra Pradesh was selected for the study, as the state ranked among top five milk producer states and contributed to the tune of 7.69% in country milk production (Animal Husbandry Statistics, 2020). The market size reached 608.1 billion in 2022 and expected to touch 1,014.3 billion by 2028, exhibiting a growth rate (CAGR) of 8.8% during 2023-2028. The livestock industry accounts for roughly 26% of the state's agricultural GDP. The state has a large network of dairy co-operatives to help support the dairy industry. There are over 7000 milk co-operative societies in the state, with an estimated membership of 8 lakh people. Four blocks were chosen at random, two each from Chittoor and Vishakhapatnam districts. They were chosen based on the districts with highest and lowest milk procurement per day respectively. One village

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was chosen at random from each selected block.

Collection of data

A complete enumeration of the selected villages was conducted, and a sample of 20 milk producer households was drawn from each village. The primary data was collected from a sample of 80 households by using well-structured schedule through personal interview. The data pertaining to socio-economic and demographic particulars of households like age, education, family composition, occupation, operational land holding, type of livestock, investment on livestock, machinery and equipment, value of feed, fodder and other miscellaneous expenses, milk production level and prices etc. were collected from respective farmers.

Analytical Framework

In order to achieve the objectives of the study, the data gathered from 80 dairy farmers have been screened, tabulated and analysed using different techniques, which have been discussed in the following sections.

Cost and returns of milk production

Milk production costs and returns are important indicators of profitable dairy farming. Fixed and variable costs combine to constitute the total cost of milk production. To estimate the cost and returns, a budgeting technique was used.

Fixed cost

Fixed costs are costs that do not fluctuate with the level of output and remain constant in the short run. Depreciation of assets and the interest rate on fixed capital are two components of fixed cost. The fixed cost was calculated using the capital recovery cost (CRC) method. The CRC method was used to calculate depreciation cost, which is the annual payment that repays the cost of fixed assets over the asset's useful life, resulting in an economic rate of return on investment.

The formula for the estimation of CRC is:

$$R = Z\left[\frac{(1+r)^n r}{(1+r)^n - 1}\right]$$

Where, R is the capital recovery cost, Z is the initial value of the capital asset, r is the interest rate, n is the useful life of the assets. The duration of assets' usefulness were estimated to be 50 years for the pucca cattle shed, ten years for the kutcha shed, six years for the manual chaff cutter, and ten years for the motorized chaff cutter. Milch animals' useful lives varying on the type of animal, and were

estimated to be 10, 8, and 10 years for local cows, crossbred cows, and buffalo, respectively. The total CRC was then allocated to each individual animal using the Standard Animal Units (SAUs). When an asset was purchased with borrowed capital, the actual interest charged by the bank was used as 'r,' whereas when own funds were used, the interest rate on a 1-5year term deposit was used.

Variable cost

Variable costs are the costs incurred on different production factors and which can be modified in the short term. Three items, include feed cost, labour, veterinary and miscellaneous costs.

Feed and fodder cost

It includes cost of feeding dry fodder, green fodder and concentrate feed. The costs were calculated as a product of the quantity fed to animals and the price of each feed. The prices of farm harvest in the study region were considered for home grown feed and fodder. The imputed value of the crop was adopted at the village price of a certain type of forage, in particular green feed where agricultural harvest prices are not available. The cost of concentrate (made at home) was determined by taking into account the composition and the market prices of each ingredient.

Labour cost

During the personal interviews with the farmers, information about labour requirements for various farm operations and labour costs was gathered. The cost of labour comprised both family labour and hired labour. Hired labour costs were calculated based on the type of work assigned and wages paid, whereas family labour costs were calculated based on the existing wage rate of permanent farm labour.

Veterinary and Miscellaneous expenses

The costs of breeding and healthcare were covered by the veterinary costs. The costs of insemination by artificial means (AI), natural service, vaccination, medicines, veterinary fees and other related expenses were included. Various expenses included repair expenses for fixed assets, water, electricity, insurance and other incidental charges. These were joint costs, and the distribution was therefore carried out on the basis of the standard animal units (SAU).

Standard animal units (SAUs):

The dairy animals were converted into SAUs using the factors suggested by Sirohi et al. (2015) for the southern region.

Other cost concepts used

***Gross cost*:** It was obtained by adding all the cost components including fixed cost and variable cost.

Goss cost = Total variable cost + Total fixed cost

Net cost: The net cost was worked out by deducting the imputed income earned through dung, from the gross cost.

Net cost = Gross cost – Value of dung

***Gross returns*:** Gross returns were obtained by multiplying milk yield of an individual milch animal with respective prevailing prices in the study area.

Gross returns = Quantity of milk × Market price of milk

Price of milk: The price of milk differs among different categories of animals. Therefore, weighted average price of milk was taken as an explanatory variable in the marketed surplus function and calculated for each household as follows:

Weighted average price= "PiWi / "Wi Where,

Pi is price per litre of the ith type of milk

Wi is total quantity of ith type of milk sold by the household

***Net returns*:** A net return was calculated by subtracting net cost from gross returns.

Net returns = Gross returns – Net cost

All the costs were calculated for per SAU per day. This also indicates the cost of maintaining an animal per day.

Results and Discussion

The cost and return analysis is regarded as a vital aspect of milk production since it indicates the profitability of enterprise besides enabling better decision-making process.

Costs and returns of milk production for indigenous cow

Table 1 indicates the total costs and returns of milk production for indigenous cow in the study area. The percent of fixed cost to the gross cost was found to be lowest (11.52%) for largest herd size category. The total variable cost accounted for 88.41 per cent of overall gross cost. overall feed and fodder constituted a major share of 67.76 percent of overall gross cost. The overall average price of indigenous milk was ₹ 30 per litre, although slightly varies with herd size category. Overall cost of milk production per litre was estimated to be ₹ 32.54, which ranged between ₹ 31.42 to ₹ 33.11 for large and small category, respectively. The overall negative net returns (₹ 2.55/ litre) of milk production indicating that indigenous cows are not economical. Nonetheless, farmers are rearing indigenous cow mainly for two reasons, i.e., first, own consumption of milk as they considered indigenous cow milk is better than crossbred and health benefits particularly to the children, secondly, ease of rearing– can be reared with locally available resources. Hence, scientists need to work on improvement in productivity of indigenous cows through breeding and farmers need to adopt better management practices. The result obtained with regards to the cost and returns of milk production in the case of indigenous cows were found to be in conformity with the earlier study conducted by Priya (2018) and Vanishree (2018)

Costs and returns of milk production for crossbred cows

Results provided in Table 2 indicates that the overall total fixed cost per day contributes about 12.07 per cent of overall gross cost. The total fixed cost was per animal was found vary with herd size categories. The share of total variable cost to the gross maintenance cost was 87.93 per cent. The overall feed and fodder

Table 1 Costs and returns of milk production for indigenous cow (₹/animal/day)

Cost/ Returns Components	Herd size category			Overall
	Small(1-5SAU)	Medium(6-9SAU)	Large(>9SAU)	
Total Fixed Cost (TFC)	20.40(11.63)	22.14(11.62)	24.33(11.52)	22.29(11.59)
Dry fodder (F1)	13.99(7.98)	15.21(7.99)	17.15(8.12)	15.45(8.03)
Green fodder (F2)	53.45(30.48)	57.21(30.04)	63.79(30.20)	58.15(30.24)
Concentrate (F3)	50.87(29.01)	56.36(29.59)	63.10(29.88)	56.78(29.49)
Feed and fodder cost (V1=F1+F2+F3)	118.30(67.47)	128.78(67.61)	144.03(68.20)	130.37(67.76)
labour cost (V2)	19.96(11.38)	21.45(11.26)	22.88(10.83)	21.43(11.16)
Miscellaneous (V3)	16.69(9.52)	18.10(9.50)	19.96(9.45)	18.25(9.49)
Total Variable Cost (TVC=V1+V2+V3)	154.95(88.37)	168.33(88.38)	186.87(88.48)	170.05(88.41)
Gross Cost (A=TFC+TVC)	175.35(100.00)	190.47(100.00)	211.19(100.00)	192.34(100.00)
Value of Dung (B)	2.90	3.27	3.56	3.24
Net Cost (C=A-B)	172.45	187.20	207.63	189.09
Price of milk (₹ /litre)	29.27	30.03	30.39	29.90
Average milk production (litre/animal/day) (E)	5.21	5.70	6.61	5.84
Gross Return (D)	152.45	171.25	200.83	174.84
Net Returns (D-C)	-19.99	-15.95	-6.80	-14.25
Cost of milk production (₹ /litre) (C/E)	33.11	32.83	31.42	32.45
Returns (₹ /litre)	-3.84	-2.80	-1.03	-2.55

**Figures in parentheses indicate percentage of gross cost*

cost constituted a major portion of about 75.64 per cent of the gross cost. Among the feed and fodder, the green fodder occupied a major share of 48.58 per cent in the gross cost, followed by concentrates (43.04%) and dry fodder (8.38%). The overall labour cost was estimated to be ₹ 29.35 per day with a share of 13.67 per cent to the gross cost and it was found to be highest for small herd size category (14.31%) and lowest in case of large herd size category (12.93%).

The average price of milk was estimated as ₹ 25.33, ₹ 26.07and ₹ 26.45 for small, medium and large herd size category respectively. It is worth mentioning that market prices of crossbred milk are lower than indigenous cow and buffalo milk. This is because of the fact that crossbred cow milk has comparatively lower fat content. The milk of indigenous cow is being preferred by the consumers particularly children and old age persons because of health properties. There is a nudging effect on the customers that indigenous milk is A2 which is better than A1 milk (crossbred).

The overall cost of production was estimated to be ₹ 22.64 per litre of milk. Overall net return was estimated to be ₹ 3.19 per litre of milk and also found to be positive for all the categories of dairy households. Interestingly, despite the lower per liter price of crossbred milk, rearing of crossbred is an economically viable enterprise. This is because of higher productivity. The result obtained regarding the cost of production and net return per litre of milk for crossbreed were similar with the previous studies conducted by Priya (2018) and Vanishree (2018).

Costs and returns of milk production for buffalo

Table 3 shows that, the share of variable cost to the overall gross maintenance cost was found to the tune of 89.74 per cent. It is clear from the Table 3 the contribution of overall feed and fodder cost to the gross cost was found to be 67.40 per cent which varied from 66.15 per cent in small herd size to 68.74 per cent in case of large herd size category. Among the feed and fodder, the contribution of overall green fodder, dry fodder and concentrates to the gross cost was worked out to be 34.43 per cent, 5.83 per cent and 27.14 per cent, respectively. The overall labour cost was estimated to be ₹ 42.11 per day with a contribution of 15.86 per cent to the gross maintenance cost. It can be interpreted that the

Table 2 Costs and returns of milk production for crossbred (₹ /animal/day)

Cost/ Returns components	Herd size category			Overall
	Small (1-5 SAU)	Medium (6-9 SAU)	Large (>9SAU)	
Total fixed cost (TFC)	28.53	29.53	31.33	29.46
	(12.37)	(11.80)	(11.91)	(12.07)
Dry fodder (F1)	12.97	13.65	14.85	13.60
	(8.58)	(8.15)	(8.36)	(8.38)
Green fodder (F2)	76.61	78.66	84.02	78.88
	(50.67)	(46.93)	(47.27)	(48.58)
Concentrate (F3)	61.61	75.29	78.87	69.89
	(40.75)	(44.92)	(44.37)	(43.04)
Feed and fodder cost	153.19	167.60	177.75	162.37
(V1=F1+F2+F3)	(74.81)	(75.93)	(76.72)	(75.64)
Labour cost (V2)	28.92	29.53	29.96	29.35
	(14.31)	(13.39)	(12.93)	(13.67)
Miscellaneous (V3)	21.99	23.61	23.96	22.95
	(10.88)	(10.69)	(10.34)	(10.69)
Total variable cost	202.09	220.74	231.67	214.67
(TVC=V1+V2+V3)	(87.63)	(88.20)	(88.09)	(87.93)
Gross cost (A=TFC+TVC)	230.62	250.26	263.00	244.13
	(100.00)	(100.00)	(100.00)	(100.00)
Value of dung (B)	2.78	3.27	3.56	3.11
Net cost (C=A-B)	227.84	246.99	259.54	241.02
Price of milk (₹/litre)	25.33	26.07	26.45	25.82
Average milk production (litre/animal/day) (E)	10.35	10.63	11.31	10.76
Gross return (D)	262.17	276.96	299.09	275.01
Net returns (D-C)	34.33	29.97	39.65	33.99
Cost of milk production (₹ /litre)(C/E)	22.01	23.25	22.94	22.64
Returns (¹ /litre)	3.32	2.82	3.51	3.19

**Figures in parentheses indicate percentage of gross cost*

Table 3 Cost and returns of milk production for buffalo (₹ /animal/day)

Cost/ Returns components	Herd size category			Overall
	Small(1-5 SAU)	Medium (6-9 SAU)	Large (>9 SAU)	
Total Fixed Cost (TFC)	26.93(10.82)	26.66(10.07)	28.15(9.93)	27.25(10.27)
Dry fodder (F1)	14.30(5.74)	15.82(5.97)	16.34(5.77)	15.49(5.83)
Green fodder (F2)	85.40(34.29)	91.36(34.50)	97.80(34.51)	91.52(34.43)
Concentrate (F3)	65.01(26.11)	71.07(26.84)	80.67(28.47)	72.25(27.14)
Feed and fodder cost (V1=F1+F2+F3)	164.71(66.15)	178.25(67.31)	194.81(68.74)	179.25(67.40)
Labour cost (V2)	39.90(16.02)	42.55(16.07)	43.89(15.49)	42.11(15.86)
Miscellaneous (V3)	17.47(7.02)	17.36(6.56)	16.55(5.84)	17.13(6.47)
Total Variable Cost (TVC=V1+V2+V3)	222.08(89.18)	238.16(89.93)	255.25(90.07)	238.49(89.73)
Gross Cost (A=TFC+TVC)	249.01(100.00)	264.82(100.00)	283.40(100.00)	265.74(100.00)
Value of Dung (B)	6.20	8.70	11.20	8.70
Net Cost (C=A-B)	242.81	256.12	272.20	257.04
Price of milk (₹/litre)	37.23	38.40	39.02	38.22
Average milk production (litre/animal/day) (E)	7.71	8.49	9.29	8.50
Gross Return (D)	287.14	326.03	362.61	325.26
Net Returns (D-C)	44.33	69.91	90.41	68.22
Cost of milk production (₹/litre) (C/E)	31.48	30.17	29.29	30.31
Returns (₹/litre)	5.75	8.23	9.73	7.90

**Figures in parentheses indicate percentage of gross cost*

maintenance cost can be reduced by effective management of locally available feed, fodder and labour, etc. The average price of milk was estimated as ₹ 37.23, ₹ 38.40 and ₹ 39.02 for small, medium and large herd size category respectively (Table 3). The prices of buffalo milk are marginally higher than the indigenous milk and crossbred milk. Although buffalo milk has comparatively higher fat content but due to less developed market infrastructure, farmers could not realized better prices of milk in general and buffalo milk in particular. The per litre cost of milk production was found to be negatively related with the herd size due to the economics of scale. The net return was found to be ₹ 5.75, ₹ 8.23 and ₹ 9.73 per litre of milk for small, medium and large herd size categories, respectively. The overall net return per litre of milk was worked out to be ₹ 7.90. It could be concluded that buffalo rearing is more profitable in the study area.

Conclusion

A comparative economic analysis of indigenous cow, crossbred and buffalo inferred that buffalo rearing is more profitable in Southern and North Coastal regions of Andhra Pradesh. Despite negative returns of indigenous breed, famers are rearing for mainly own consumption. However, net return in case of crossbred was found to be positive for all the categories dairy farm households. The return to scale effect was evident in buffalo and crossbred dairy farms. Buffalo milk fetches higher prices owing to higher fat content. The farmers still could not realized the full potential of dairying in the study area due to less developed organized market.

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RESEARCH ARTICLE

Berseem seed and fodder production for Punjab's dairy sector- A comparative economic analysis

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Abstract: The current study examines the on-farm profitability of berseem seed and fodder production in Indian Punjab from an innovative informal strategy for building a village-based forage seed enterprise for berseem (*Trifolium alexandrinum*). A comparative economic analysis of berseem seed, fodder, and wheat production in *rabi* season, 2019-20 found that berseem (as seed and fodder) was more profitable than wheat. Thus, there is a need to shift some area to berseem, particularly its seed production, which will not only help dairy producers increase their income but will also provide nutritious fodder for milch animals to reach their full potential. Quality seed production of improved varieties of berseem will also address the issue of recommended high-yielding varieties being unavailable during the peak sowing season. Berseem seed disposal patterns suggested that practically all seed produced by farmers was sold in the same year (without any certification) due to its high demand. Around 90% of growers reported that the primary challenge they faced during the production and marketing of berseem seed was high price volatility and losses during cleaning and grading, followed by an insufficient supply of high-quality seed (85%), a lack of an appropriate marketing channel (80%), and an inadequate price for the produce (80%). The primary challenge for berseem fodder growers was high labour costs (28%). Creating awareness among farmers/livestock keepers/policymakers about new berseem varieties, providing incentives, and ensuring a market for seed production will all contribute to increasing demand for quality fodder and, thus, seed production of berseem. The government should stabilize input and output prices, which can contribute significantly to sustaining higher productivity and the livelihoods of Punjab's dairy farmers, resulting in more effective rural development and poverty reduction.

Keywords: Berseem seed, cultivation, dairy, fodder, returns, wheat

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Introduction

Dairy farming has been an indispensable activity throughout the history of human civilization. It is not only economically significant but also nutritionally significant as a source of alternative food for the world's ever-growing population. Nutritious feed is critical for milch animals to reach their full potential. Among all fodder crops, berseem is the most effective at increasing milk production in lactating animals and crossbred cattle. The cost of feeding milch animals can be reduced by using berseem in place of mineral concentrate (Kumar et al. 2021; Akila and Lakshmi, 2020). An all-berseem ration is adequate for milch animals yielding up to 6-7 litres of milk daily (PAU, 2021). Berseem is the highly beneficial winter forage crop in India, which is known as 'King of Fodders' due to its highest tonnage capacity with no toxic effects. It is the most palatable fodder, due to its succulence and high nutritious value for livestock. Being a leguminous crop, berseem fodder has higher protein content than non-leguminous fodders.

Though it is not possible to easily increase the area under fodder crops, various steps can be taken to enhance their productivity and hence, production. Out of different inputs, the cultivation of fodder crops for the production of seed particularly the quality seed by replacing the area with other crops can play an important role to increase the fodder production in the country. Seed yield in forage crops is generally low due to more vegetative growth as well as reduced seed setting. Lack of quality seed availability results in less fodder production which affects livestock

production. In developing countries, fodder production has many folds restricted by land dearth, the inadequacy of standard seed (Tufail et al.2019), and unawareness about fodder production technology and usage (Kamanzi and Mapiye, 2012).

In India, small farmers follow a mixed crop-livestock farming system which constitutes the major part of agricultural productivity. The livestock sector plays a fundamental role in the Indian economy by employing about nine percent of the population (Bhardwaj et al. 2020). The timely availability of seed to the farmers helps in timely sowing and realization of its full potential as fodder (Canbolat et al. 2006) and thus enhancing the income from dairying. With an attainable seed yield of (0.4 t/ha) and the seed multiplication ratio (SMR) of 20, only 6.4 percent of the total seed requirements of berseem can be met at the national level. During 2015-16, 10,375 MT berseem seed was imported to fill the gap in production and demand (Chauhan et al. 2017). Among the forage crops, berseem seed import is highest with 0.03-0.04 percent share of total imports into India which turns out to be around 20 million US dollars. To reduce the loss to the exchequer in the form of foreign exchange, there is a need to reduce the seed import from abroad. Thus, suitable technological and appropriate action points are needed to increase berseem seed production. Because of its high-yielding nature, huge demand for berseem seed exists in the market. During the peak sowing period, there is a shortage of berseem seed in the market, and farmers are obliged to buy unrecommended low-yielding varieties.

Punjab state is among the leading states producing berseem with wheat being the main competing *rabi* season crop. The state has about one-fourth of the total area of fodder crops and livestock contributes approximately forty percent (40%) to the annual income of small farmers. The current livestock population of the state is 81.2 lakh (62.4 lakh adult) with a fodder supply of 31.4 kg per animal per day which is far from satisfactory. Based on 40 kg green fodder per adult animal per day, approximately 911 lakh tonnes of fodder is required (PAU, 2021). In this backdrop, the present study was carried out to study the comparative cost of production of Berseem seed and wheat crop to study the share of different inputs involved along with measures to promote berseem seed production in the state.

Materials and Methods

The present study was conducted in the Punjab state during the year 2019-20. A widely distributed and representative sample was drawn by using multistage sampling technique with selection of district at the first stage (Ludhiana and Gurdaspur), followed by selection of one block from each selected district at the second stage (Dehlon from Ludhiana and Dina Nagar from Gurdaspur district), villages at third stage and farmers at the fourth stage of sampling. A sample of 80 berseem fodder-cum-wheat growers (40 from each district) was randomly selected without replacement representing different farm size categories to study the economics of berseem fodder and wheat cultivation (Table 1). In addition, to study the economics of berseem seed production, 20 berseem seed producers were also taken from the selected districts as very few farmers follow the practice of fodder seed production. From the selected 20 seed growers, 17 were large (>10 ha) and medium farmers (4-10 ha); three respondents were small farmers (1-2 ha) and none was from the semi-medium and marginal farm size category. Thus, for comparative economic analysis of berseem fodder, berseem seed and wheat crop cultivation, a total of 100 respondents were selected for the study.

The data were collected by personal interview method on a well-structured and pre-tested survey schedules regarding different inputs used, returns obtained, seed marketing pattern and problems faced by the growers. To study the trend in the area under fodder crops especially berseem, the time series secondary data on the area under fodder crops in Punjab was also taken from the Department of Animal Husbandry, Punjab. The compound annual growth rates (CAGRs) of total cropped area, the area under fodder crops, and area under berseem crop were estimated for Punjab for the five periods viz. Period I: 1981-1990, Period II: 1991-2000, Period III: 2001- 2010, Period IV: 2011-2019, and Period V: 1981-2019. The growth model adopted is as under:

$$Y_t = AB^t$$

Where,

Y_t = Total cropped area/area under fodder crops/area under berseem crop for the year 't'.

t = Time variable (1,2…, n) for each period.

A = Constant

Log transformation of the above function is:

Table 1 Sampling design of berseem seed, berseem fodder, and wheat growers in Punjab

(No. of farmers)

Particulars	Farm size category					Total
	Marginal (<1ha)	Small (1-2 ha)	Semi-medium (2-4 ha)	Medium (4-10 ha)	Large (>10 ha)	
Berseem fodder and wheat growers	12	19	26	19	4	80
Berseem seed producers	0	3	5	5	7	20
Total	12	22	31	24	11	100

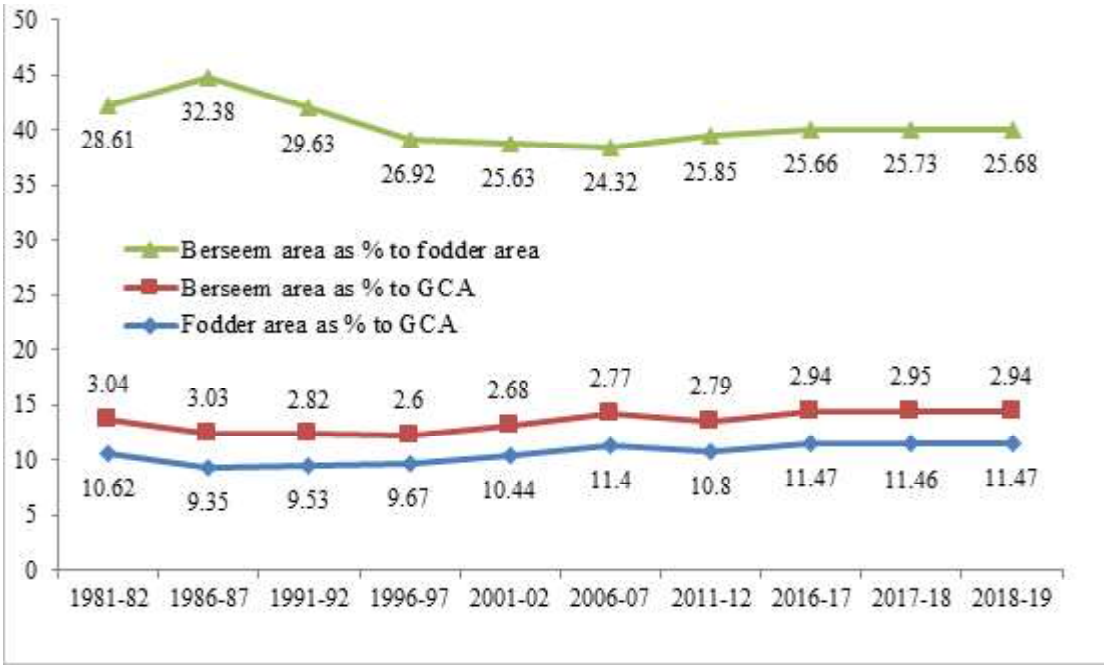


Fig. 1 Trends in area under fodder crops in Punjab

$\ln Y_t = \ln A + t (\ln B)$.

Where,

$\ln B = \ln (1+r)$, and

$r = [\text{antilog} (\ln B) - 1]$

CAGR (%) $= [\text{antilog} (\ln B) - 1] \times 100$

The student's t-test was used to test the significance of CGRs.

Results and Discussion

Berseem is one of the major *rabi* forage crops in Punjab state and occupies the maximum area among forage crops during the winter season. Though the area under different fodder crops increased from 10.62 percent of total cropped area (TCA) in 1981-82 to 11.47 percent in 2018-19, the percent share of berseem in the TCA decreased from 3.04 to 2.94 during this period, according to the analysis (Figure 1). Despite its benefits to animal nutrition, berseem's percentage in total fodder fell from 28.61 to 25.68 percent, owing to a rise in the area under key cereal crops such as rice and wheat. Another major factor was the extensive mechanization of state agriculture, which resulted in a significant decrease in the number of draught animals. Due to the introduction of high yielding quality cultivars, the area under berseem crop as a percentage of TCA increased somewhat in 1986-87 but then began to decline. The compound annual growth rates (CAGRs) of TCA and area under fodder crops were calculated to be 0.34 and 0.60 percent, respectively, from 1981-82 to 2018-19, but the area under berseem crop grew at a negligible rate (Table 2). The CAGR of the berseem crop was highest from 1981-82 to 1990-91, after which it began to decline. Thus, from 1981-82 to 2018-19, the area under berseem crop stayed nearly constant. TCA, fodder crop area, and berseem crop area all increased, although not in the same way. Due to insufficient area

Table 2 Growth in area under total crops, fodder crops and berseem crop in Punjab

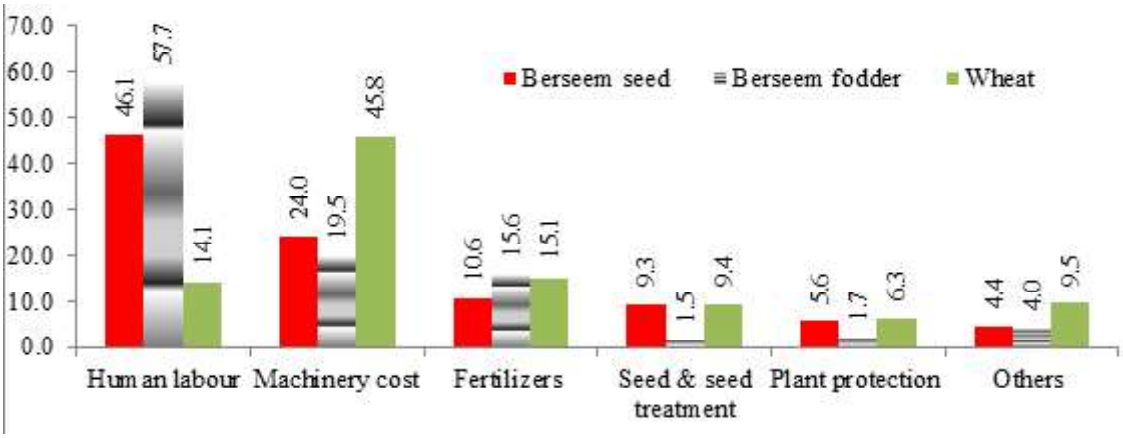
Period	Total cropped area		Area under total fodder crops		Area under berseem crop	
	CAGR	R^2	CAGR	R^2	CAGR	R^2
1981-82 to 1990-91	1.01***	0.97**	0.55NS	0.04NS	2.06*	0.27NS
1991-92 to 2000-01	0.64^{***}	0.94^{**}	-0.04^{NS}	0.001^{NS}	-1.56^{NS}	0.62^{**}
2001-02 to 2010-11	-0.04^{NS}	0.03^{NS}	-0.269^{NS}	0.01^{NS}	0.02^{NS}	0.001^{NS}
2011-12 to 2018-19	-0.11^{NS}	0.60^{**}	0.82^{***}	0.98^{NS}	0.87^{***}	0.91^{**}
1981-82 to 2018-19	0.34^{***}	0.70^{**}	0.60^{***}	0.48^{**}	0.04^{NS}	0.004^{NS}

Source: Department of Animal Husbandry, Punjab

***, ** and * Significant at 1, 5 and 10percent level of significance; NS: Non-significant

CAGR: Compound annual growth rate (percent per annum)

Fig 2 Percent share of different inputs in total variable costs



Note: Others include cost of irrigation, marketing, transportation etc.

Table 3 General characteristics of the selected respondents

Category	Berseem fodder and wheat growers (n=80)	% to total	Berseem seed producers (n=20)	% to total
		Age (Years)		
Up to 40	29	36.25	3	15.00
40-50	36	45.00	10	50.00
>50	15	18.75	7	35.00
Total	80	100.00	20	100.00
		Education level		
Illiterate	6	7.50	-	-
Up to middle	11	13.75	-	-
Matric	34	42.50	2	10.00
10+2	17	21.25	11	55.00
Graduate	12	15.00	4	20.00
Postgraduate	-	-	3	15.00
Total	80	100.00	20	100.00

under fodder crops, there is a demand-supply mismatch for fodder. As a result, there is a pressing need to expand the area under fodder and berseem crops.

General information about the respondents

According to the survey, 50 percent of the berseem seed growers were between the ages of 40 and 50, with 35 percent being over 50 and the remaining 15 percent being the age of up to 40 years (Table 3). Similarly, the majority of berseem fodder and wheat growers, 45.00 percent, were in the age category of 40 to 50 years, with about 36 percent belonging to a younger age group of up to 40 years, and about 19 percent belonging to a considerably older age group of more than 50 years. The level of education aids farmers in gaining more understanding about farming operations to make their vocation more profitable. The majority of seed growers (55%) had completed secondary school education, with the remaining 35 percent having completed a bachelor's degree or higher. This suggests that, as a result of their education, the respondents were well aware of the importance of high-quality seed and its advantages. On the other hand, roughly 43 percent of berseem fodder and wheat growers were matriculated, 21 percent had completed 10+2, 13.75 percent were in the middle class, 15 percent were graduates, and only 7.50 percent were illiterate.

The average size of total operational holding for the selected berseem seed growers was 6.01 hectares, of which about 67 percent was owned, some of the farmers took rental land for cultivation in addition to owned operational land (46%), and about 13 percent of the seed growers had leased out their land. The average operational holding of berseem fodder/wheat producers was 3.92 hectares, with the majority of the farmers having owned land (68.98%), followed by leased in (43.27%) and leased out (12.35%) holdings (Table 4). Wheat occupied the majority of the cropped area for both berseem seed (29.28%) and fodder growers (34.44%) during the *rabi* season, and the area under berseem seed and fodder production was 12.36 and 5.95percent, respectively (Table 5). This happened as the farmers that opted for berseem seed production were mostly large farms.

Comparative economics of berseem (seed and fodder) and wheat cultivation

It's crucial to examine the relative cost-return structures of berseem seed, berseem fodder, and wheat production. The results of the analysis of various components of the operational cost of berseem cultivation for seed and fodder production as well as wheat crop production are presented in Table 6 and Figure 2. The total operational costs per hectare for the berseem seed crop was Rs. 51312. Human labour, at Rs. 23649 (46.1%), occupied the largest share of the operational costs for seed production in the current study, followed by machinery costs at Rs. 12298. (24%). Apart from these expenditures, fertilizer, which includes urea, DAP, SSP, MOP, and potassium nitrate, had an 11 percent share. Some berseem seed growers used phosphorus in the form of DAP, while others used SSP. Along with phosphorus, SSP fertilizer contains about 12-14 percent sulphur, which is beneficial to the berseem crop. Farmers were observed to be applying 7.38 kg of potassium nitrate per hectare after the last cut of berseem fodder to increase berseem seed production. The cost of seed and seed treatment was Rs.4595 (9.3%), whereas the cost of plant protection measures was Rs.2880 (5.6%). Farmers in Punjab applied just approximately 8 irrigations to berseem seed crop due to ample rain in the year 2019-20, and farmers bore the expense of irrigation of Rs. 1075, which accounted for only 2.1 percent of total variable cost. Irrigation costs included maintenance costs, owing to the fact electricity supply is free to the farm sector in the state. The total variable expenses for berseem fodder production came out to Rs. 33733 per hectare. Because of the enormous labour involved in taking several cuttings for feeding the livestock, human labour had a substantial part of roughly 58 percent (Rs. 19458/ha) among the various inputs. Another significant factor was the usage of machinery, which accounted for Rs 6580 (19.5%), followed by fertilizers (15.6%), plant protection (1.7%), irrigation (1.6%), and seed & seed treatment (1.5%).

In the case of wheat, the total operational costs per hectare were around Rs. 33723. Among the various components of operational costs, machinery costs accounted for the greatest proportion, at Rs. 17105 (45.8 percent), due to mechanical harvesting and threshing. The cost of human labour was Rs. 5250, accounting for approximately 14 percent of total variable costs. Apart from these costs, fertilizer accounted for around 15 percent of the total, followed by seed and seed treatment (9%), and plant protection measures (6.3%). Farmers bore a cost of Rs. 1430 for ten irrigations to the wheat crop, accounting for only 3.8 percent of total variable costs. Marketing costs associated with unloading and cleaning, as well as transportation, accounted for 3.3 percent of total variable costs. From the analysis berseem seed production was found to be highly profitable on Punjab farms, with gross returns of Rs. 177080 per hectare from the production of roughly 5.4 quintals of seed (Rs 86,000) and 700 quintals of fodder as a byproduct (Rs. 91000). Berseem seed and fodder produced returns of Rs. 142300 per hectare which includes Rs. 128700 from fodder production and Rs. 13600 from seed produced as a byproduct. On the other hand, the gross returns earned from the cultivation of wheat crop came out Rs. 115453 from grains and wheat straw, which were much lower than those from berseem seed and fodder (Table 7). The berseem seed production has higher variable costs (Rs. 51312/ha) than berseem fodder production (Rs. 33733/ha) and wheat (Rs. 37338/ha). However, it yields higher returns over variable costs (Rs. 125688/ha) than berseem fodder (Rs. 108577/ha) and wheat (Rs. 78115/ha). This points towards the profitability of berseem seed production in comparison to its competing berseem fodder crop and wheat crop during the *rabi* season. The benefit-cost ratios for berseem seed, berseem fodder, and wheat were calculated to be 3.4, 4.2, and 3.1, respectively, implying a modest increase in net revenue for berseem seed growers relative to wheat producers. As a result, some *rabi* crops area especially wheat crop area must be shifted to berseem seed production to increase farm profitability and meet the state's demand for high-quality berseem seed.

Table 4 Size of operational holding of the sampled respondents in Punjab (ha/farm)

Particulars	Berseem fodder-cum-wheat growers	Berseem seed producers
Owned land	2.70	4.03
Leased in land	1.70	2.78
Leased out land	0.48	0.80
Total operational holding	3.92	6.01

Note: Figures in parentheses indicate percentages to the total operational holding of the respective category

Table 5 Area under berseem crop at the sampled farms in Punjab

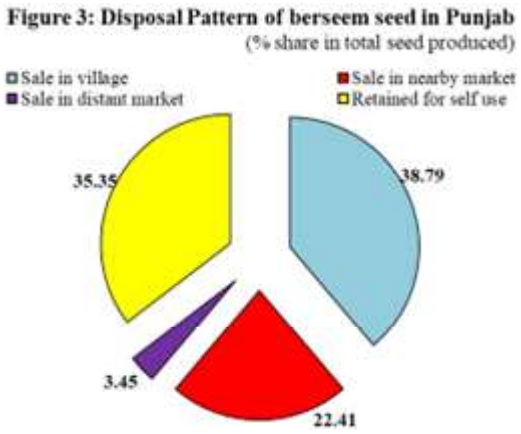
Respondents	Area (ha/farm)		% Share in total cropped area	
	Berseem	Wheat	Berseem	Wheat
Berseem fodder growers	0.45(95.69)	2.57	5.95	34.44
Berseem seed producers	1.46(100.0)	3.46	12.36	29.28

Figures in the parentheses indicate the percent share of the berseem crop in total *rabi* fodders

Table 6 Comparative cost of cultivation of berseem (seed and fodder) and wheat in Punjab (Per ha)

Sr. No.	Inputs	Berseem				Wheat	
		Seed		Fodder			
		Quantity	Value (Rs)	Quantity	Value (Rs)	Quantity	Value (Rs)
1	Seed & seed treatment						
	Seed (kg)	25	4595	30.6	483	100	3250
	Chlorpyriphos 20 EC (ml)	-	-	-	-	400	120
	Raxil Easy (ml)	-	-	-	-	32.5	155
	Rhizobium (Packet)	2.5	100	0.7	28	-	-
	Bavistin (gm)	70	75	-	-	-	-
	Subtotal	-	4770	-	510	-	3525
2	Fertilizers (kg)						
	Urea	143.4	850	4.7	1288	225	1335
	DAP	138.8	3330	73.9	438	137.5	3300
	Superphosphate	62.7	470	144.1	3338	-	-
	Muriate of potash	15.6	283	19.6	148	50	1000
	Potassium Nitrate	7.4	518	44.2	40	-	-
	Subtotal	-	5450	-	5250	-	5635
3	Plant protection		2880	-	585	-	2335
4	Irrigations (No.)	7.5	1075	3.8	536	10	1430
5	Human labour (hours)	364	23649	389	19458	105	5250
6	Machinery cost						
	Tractor (hours)	18.3	12298	10.7	6580	15	8355
	Combine harvester & straw reaper	-	-	-	-	-	8750
	Subtotal	-	12298	-	-	-	17105
7	Marketing & transportation charges	-	62	-	62	-	1236
8	Interest on variable costs @9% p.a. for half the crop period	-	1129	-	742	-	822
9	Total variable costs (1 to 8)	-	51312	-	33723	-	37338

Unlike berseem seed, wheat has assured marketing in the state at a minimum support price. Therefore, the disposal pattern for berseem seed produced by respondents was examined (Figure 3). The sample respondents produced a total of 232 quintals of berseem seed. Out of this, 90 quintals (38.97%) were sold directly to seed consumers, such as fellow farmers and relatives, while 80 quintals (35.35%) were retained for farmers' own use. The remaining 52 quintals (22.41%) were sold to seed dealers and distributors. Additionally, eight quintals (3%) were sold in distant marketplaces in neighboring states. Due to the great demand for seed, practically all of the seed produced by farmers was sold in the same year.



Issues confronting berseem seed farmers

Various difficulties were encountered by seed growers throughout the production and marketing of berseem seed (Figure 4). The severity of challenges encountered by farmers varies according to producer and variety. The most frequent issue

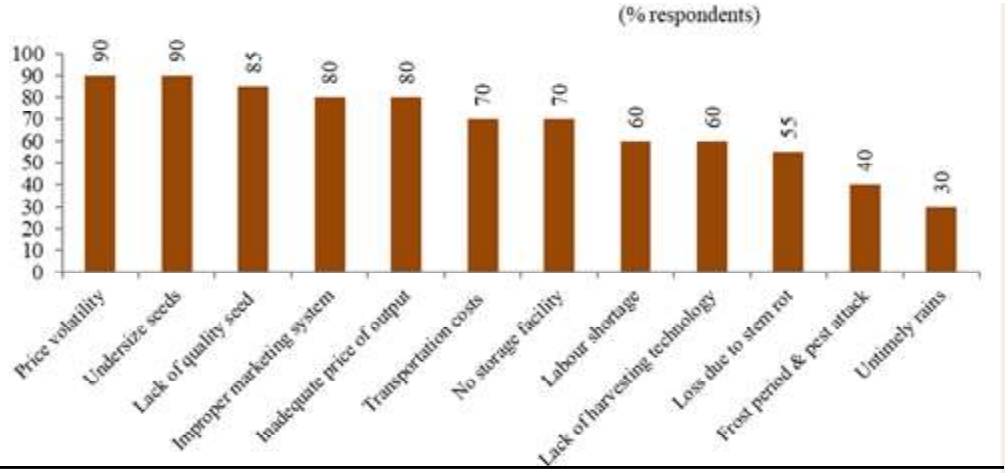


Fig. 4 Issues confronting berseem seed farmers (% respondents)

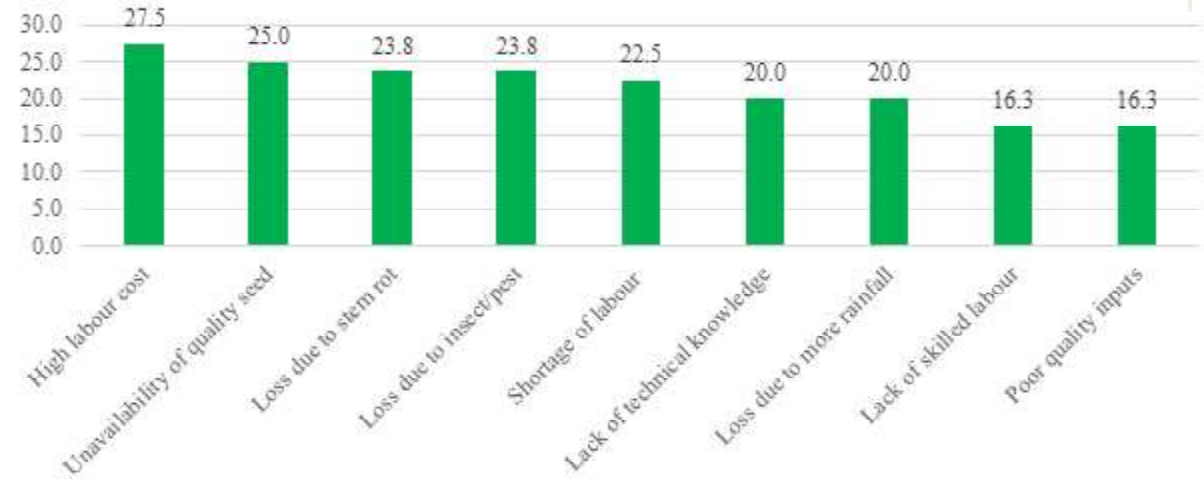


Fig. 5 Problems faced by the berseem fodder growers (% respondents)

Table 7 Returns from the cultivation of berseem (fodder and seed) and wheat in Punjab (Per ha)

Particulars	Berseem				Wheat	
	Seed Quantity	Value (Rs)	Fodder Quantity	Value (Rs)	Quantity	Value (Rs)
Main Product (q)	5.38 (seed)	86080	990 (fodder)	128700	53.75 (grain)	103469
By Product (q)	700 (fodder)	91000	0.85 (seed)	13600	36.88 (straw)	11984
Gross returns	-	177080	-	142300	-	115453
Returns over variable costs	-	125688		108577		78115
B:C ratio	3.4	4.2	3.1			

encountered by over 90 percent of producers was high price volatility throughout the marketing of berseem seed, which rendered growers unable to forecast the actual price of the output (Figure 4). Almost 10-15 kg of seed is wasted during cleaning and grading in the form of broken seeds, undersized seeds, and so on, and approximately 90 percent of respondents experienced this issue. According to about 85 percent of respondents, the supply of high-quality seed was insufficient in comparison to the demand in Punjab.Apart from these concerns, almost 80 percent of respondents reported a lack of an adequate marketing channel. Another 80 percent of respondents reported having difficulty in pricing their produce because the BL 1 and BL 10 varieties are identical in size, shape, and colour, making differentiation impossible. While BL 10 have more price than BL 1, its producers received the same price as BL 1's producer. Additionally, farmers faced challenges such as high transportation costs and a lack of storage space (70% each); labour shortages during peak harvesting periods and a lack of appropriate technology for seed harvesting (60% each); stem rot disease loss (55%); frost and insect/pest damage (40% each); and heavy rainfall (30%).

Problems reported by the berseem fodder growers

The state's berseem fodder growers face a variety of problems, as presented in Figure 5. Around 28 percent of berseem fodder growers expressed concern about high labour expenses associated with berseem fodder growing and selling. Berseem fodder production is extremely susceptible to frost damage and is especially vulnerable to stem rot disease and insect/pest assault during February and March. As a result, adequate technical advice is required throughout the production period. Around 24 percent of farmers have run into this difficulty. Additionally, growers faced labour shortages during peak harvesting periods (22.50%), crop loss due to increased rainfall (20%), lack of high-quality seed (18.75%), lack of skilled labour (16.25%), and lack of high-quality inputs such as pesticides, fertilizers, and so on (16.25%).

Conclusions and policy implications

Berseem and wheat are two competitive crops grown in the state during the rabi season. Berseem is in high demand in the state due to the large consumption of dairy products and the multiple benefits it provides to dairy animals. Berseem cultivation in the form of seed and fodder is more profitable than wheat production. Considering the cattle population, demand for nutritious and high-quality fodder has been increasing and will continue to do so. Village-based fodder seed bank concept can also be introduced through the establishment of fodder seed villages/ farmers, fodder seed producer organizations/groups with facilities for seed processing and storage (Ghosh and Mahanta, 2016).Farmers should be educated by extension professionals on scientific methods of agricultural production technology, such as the use of the optimal seed rate, certified seed, sowing time, and crop care, to increase crop productivity. The government should stabilize input and output prices, which might be critical in sustaining increased productivity and livelihoods for Punjab's dairy farmers, resulting in more effective rural development and poverty reduction. Some important policy implications of berseem seed production are as under:

- Some area must be shifted to berseem cultivation, particularly seed production. This will not only increase income for berseem growers and dairy producers but will also provide nutritious fodder for milch animals to reach their maximum potential.

- Quality seed production of improved varieties of berseem will also address the issue of unavailability of high-yielding recommended varieties during the peak sowing season.

- The price of berseem seed shows high volatility in the market. The farmers can take the advantage of high prices during the sowing season by selling the seed at the time of sowing.

- There are no producer incentives or minimum support prices for the production of fodder crops or their seed. Educating farmers/livestock keepers/policy makers about improved high-yielding and nutritious varieties of forage crops, providing incentives, and ensuring a market for seed/fodder production would all significantly contribute to the rising demand for high-quality fodder and, consequently, seed production of forage crops.

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RESEARCH ARTICLE

Impact and determinants of membership in dairy cooperative society: The case of smallholder dairy farmers in Barpeta District of Assam

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Abstract: Dairy cooperatives are associated with creating opportunities for attaining higher level of market integration having implications for significant improvement in income of the dairy farmers. Given the positive impact of DCS membership from the analysis of multivariate regression the study further looks for factors that determine the decision to become membership of DCS. The analysis of the determinants based on logistic regression shows that milch animal holding, credit accessibility, availability of other services from DCS have positive relation with the membership decision, while dairy farming experience, distance to the cooperative collection centre and price of milk are negatively and significantly associated with cooperative membership. The study suggests that facilitating access to credit through dairy cooperatives to meet the financial viability, offering services to dairy farmers like fodder seed, subsidized concentrate feed, veterinary services, and establishment of more collection centre and improving market infrastructure can be instrumental in stimulating farmers' membership decision to join cooperatives. Overall, to increase farmer's membership the study recommends policies to take up more awareness programmes and trainings from time to time among the younger farmers on the beneficial effects of participating in cooperative system of dairying. The study concludes that cooperatives can be efficient in fostering wellbeing of the farmers with relatively higher income, employment and nutritional status.

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Introduction

Among various livestock activities, the dairy sub sector occupies an important position in the agricultural economy of India (Sujatha et al. 2015). India ranks the largest milk producer in the global sphere with 176.3 million tons of production during the year 2017-18 sharing 20% of the World's total milk production. The legacy of recent growth in the dairy sector has been largely attributable to the efforts made under the Operation Flood programme launched in 1970. The large scale development of milk marketing under Cooperative framework paved the way for development of the dairy sector with the launch of this programme. Studies point out that expansion of the dairy cooperatives creates opportunities for attaining higher level of market integration which could bring significant improvement in income of the farmers. Dairy cooperatives, apart from providing market opportunities for the milk produced to the members, also provide the technical inputs like provision of artificial insemination, health services and feed inputs (Meena and Jain, 2012). They have a tendency to fuel the uptake of membership in DCS on the one hand and increasing production and productivity of the farmers in the other. However, the performance and impact of cooperatives have not been uniform across regions of the country (Bardhan and Sharma, 2012). Unlike in other parts of India where cooperative farming has revolutionized livestock sector, Assam has remained largely unsuccessful in cooperative model of dairy developed undertaken as per various schemes of dairy development (Barbaruah, 2012). Given the evidence of successful process of dairy development enabled by cooperative movement in states like Gujarat, Punjab, Haryana etc., Assam has failed in its effort to yield a desired progress in the same. This can be pointed out to the factors influencing the membership of DCS. In the same way, there are limited studies demonstrating the impact of DCS membership on gains in parameters like income and nutrition. Given the gap, the present study makes an effort to find what are the various socio economic factors that drive the undertaking of membership in DCS and impact on income, employment and per-capita consumption of self-produced milk of member producers vis-à-vis their non-member counterpart.

Marketing of dairy output is a serious constraint in the development of this sector. Poor marketing facilities in the rural areas and lack of facilities for organized dairy processing have led to the proliferation of production clusters mainly in the peri-urban parts of the state (Sirohi et al. 2009). Although many of the dairy cooperative societies were formed under erstwhile schemes, such as Assam Rural Infrastructure and Agricultural Services Project, Rastriya Krishi Vikash Yojana, Assam Agricultural Competitiveness Project, many of these projects are practically lying defunct or the volume of milk procurement has significantly declined. As per official records, there are 374 numbers of dairy cooperative societies with membership of around 20 thousand milk producers in Assam, whereas only about 51 thousand litres of liquid milk are marketed by these dairy cooperative societies (NDDB, 2017-18). The government has been advocating for rural milk producers to join dairy cooperatives in order to improve the income of the farmers. Despite of all the efforts put in by the Dairy Development in Assam, the status of dairy cooperatives has not improved as most of the schemes are standing alone with meagre funding pattern and poor flexibility (Kakaty and Das, 2017). For cooperative network not being improved sufficiently even after several rounds of attempts at state level through funding from international agencies like World Bank and Govt. of India, producers have to sell their milk to the milk vendors/at milk market at a very low fixed price. Milk producers in the state need to be assured of a secure market for their highly perishable produce. Given that cooperatives provide marketing opportunities to smallholder farmer, it may have impact on augmenting farm income, employment and stimulating consumption of self-produced milk among farmers giving partly a food security at household level. Therefore the present study is undertaken to register the wellbeing prospect of dairying in dairy cooperative framework in the state of Assam.

Materials and methods

Data and Sampling Design

The survey is carried out in Barpeta district of Assam. Barpeta district is selected considering several factors such as concentration of dairy cooperatives, crossbred cattle population etc. including that of Barpeta district has been considered to have significant dairy activities and a major source of liquid milk supply to the processors as well as to the urban consumers in the state (Jafor, 2019). Due to the vibrancy in dairy activities in the district, clusters such as Bajali development block received special policy attention from the Govt. of Assam through initiation of Swarna Dhenu scheme in 2002. The Assam State Animal Husbandry and Veterinary (AH & V) Department has launched an ambitious project called Swarna Dhenu Scheme (Golden Cow Concept) with a view to upgrading the state's local and the implications of the scheme were manifold where farmers were benefitted with increased milk production. Another important reason for selection of the district is because it is based on high concentration of commercial dairy producers and with surplus milk production, one of the potential districts listed by NDDB in Assam (Kakaty and Das, 2017). The district has been selected purposively based on the share of dairy cooperative societies among the districts in Assam (20.19 percent share of total DCS available in Assam) (Directorate of Dairy Development, Assam). However, after making complete enumeration of the listed DCSs available in the district some of these dairy cooperatives are found to be functional. Six cooperatives (30 percent share in active DCS) have randomly been selected from the total active DCS operating in the Bajali development block. The selection of these DCS are such that they are non-contiguous to one another. A total of 150 registered members have been chosen randomly from the active dairy cooperatives. Similarly, another 150 non-members are selected from the same area to that of the members with consideration that they held at-least one in-milk cattle in the farm and carry almost similar socio-economic characteristics. In case where villages which do not have a single household which is not a member of the cooperatives, some non-members are retained from the nearby villages. Thus, the members and non-members together constitute a total of 300 milk producers. A range of information is collected in the enumeration that includes demographic, social, production and marketing related information. All the households are categorized into three categories, viz. small (1-3 milch animals), medium (3.1-6 milch animals) and large (6.1 and above) based on SAU[1] (Kumbhare et al. 1983). The survey is carried out during January 2019 to December 2019.

Empirical Models (Liner Regression Model: Impact of DCS on the wellbeing of sampled households and Binary Logit Model: Factors influencing the membership decisions)

Impact of DCS on the wellbeing of sampled households: Linear Regression

To assess the impacts of cooperative membership on certain indicators of household wellbeing of smallholder dairy farmers such as dairy income, per capita consumption of self-produced milk and labour hour spent in dairying (as potential for employment), regression models of the following form are estimated.

$$Y_i = \propto + \beta D_i + \varphi X_i + \delta_i, \quad i = 1,2, \ldots, N \qquad (1)$$

Where the dependent variable Y_i, is a measure of the indicators of households wellbeing in terms of dairy income (log specified), consumption of self-produced milk and employment expressed in the form of labour hour spent in dairying. The first indicator, i.e., dairy income is continuous variable transformed to logarithmic value due to reduce the impact of potential outlier observations while also being able to interpret coefficients in percentage terms. The other indicators are not log transformed due to scaling and the potential for occurrences of zeroes in them. The variable of our prime concern is D_i, which is a treatment variable for membership in registered dairy cooperative society

of the study location. For any household defining =1 for a cooperative member and =0 otherwise of the households. A rich set of control variables are included to control for heterogeneity between households of the study site. Includes various socio-economic characteristics that influence the dependent variables but not the treatment variable.

Factors influencing the membership decisions: Binary Logit Model

Binary logistic model is used to identify the factors that influence the households' membership in DCS. Two methods- *t*-test and chi-square test analysis are employed to investigate the differences between cooperative members and non-members. The dependent variable is a binary variable depending on whether a farmer has dairy cooperative membership or not. The explanatory variables taken are either continuous or binary. The binary logistic model (Gujarati, 2011) can be expressed as,

$$L_i = In[\frac{P_i}{1-P_i}] = Z_i = \beta_i X_i + U_i, \qquad i = 1,2 \dots \dots N \qquad (2)$$

Where,

$In[\frac{P_i}{1-P_i}]$ = Logit for taking cooperative membership

P_i = Probability of being a member of dairy cooperative societies

$1 - P_i$ = Probability that a farmer is not a member of dairy cooperative societies

β_i = Parameters to be estimated

X_i = Independent Variables

U_i = Random error term

The model on DCS membership decision takes the following form-

$$Z = \beta_0 + \beta_1 GENDER + \beta_2 HHSIZE + \beta_3 FARMEXP + \beta_4 LANDH + \beta_5 MILCH + \beta_6 DISTCOOP + \beta_7 PRICE + \beta_8 EDUC + \beta_9 CREDIT + \beta_{10} OFF - FARMY + \beta_{11} OTHERSERV + \mu$$

Where, β_0 =Constant

$\beta_1 - \beta_{11}$ =Co-efficient of the independent variables

μ = Error term

Description of the socio economic variables influencing the membership decisions of farmers in dairy cooperative societies

The study has considered several socio-economic variables to see the influence on their membership decision in dairy cooperative societies. These explanatory variables indicate farmers' socio-economic and demographic characteristics. The selection of these variables is guided by various theoretical and empirical studies conducted in various parts of India and elsewhere-Ahmed and Mesfin (2017); Awotide et al. (2015); Balghah (2019); Bardhan and Sharma (2012); Chagwaza et al. (2016); Debeb and Haile (2016); Fikadu et al. (2019); Njiru et al. (2015); Nugusse et al. (2012). One of the important variables such as 'education of the household head' is hypothesized to affect membership of DCS positively. Studies point out that education of the household head helps in right decision making to utilize household resources productively because of their ability to process information (Nugusse et al. 2012). Prior studies confirm that male headed households are more likely to take membership of DCS vis-à-vis their non-member counterpart for their increased role in milk marketing and relationship with various selling sources (Ahmed and Mesfin, 2017; Fikadu et al. 2019). Households with higher family size tend to have more labour supply inducing adoption of commercial dairying along with their increased propensity to participate in cooperatives model of milk marketing (Chagwaza et al. 2016; Awotide et al. 2015). However, empirical results also indicate that as household size increases, domestic consumption also shoots up implying reduced marketable milk surplus and discouraging the household to take the membership of DCS (Tefera and Wold, 2015). Cooperative membership is also influenced by the number of years a farmer is engaged in farming (Balgah, 2019). Availability of off-farm income sources may influence the membership decision both positively and negatively. According to Tefera and Wold (2015), having extra income from non-farm activities facilitates to invest more in dairy farming leading to rise in the intensity of farming operations and farm output and finally influence the farmers to choose assured sources of milk sale such as DCS through adopting membership. However, Bagher (2011) finds that with involvement in other off-farm sources of income interests to intensify dairying leads to not becoming the member of DCS.

Certain variables relating to farmers' asset ownership viz. size of land holding and milch animal holding are generally tend to have a positive relationship with cooperative membership (Awotide et al. 2015; Njiru et al. 2015). Chagwiza et al. (2016) have indicated that distance to the cooperative collection centre has a negative and significant relationship with farmers' membership to dairy cooperatives. The lesser the distance to collection point, producers' transportation hindrances are reduced and likely to incentivize the farmers to adopt membership. The other independent variables assumed to have positive relationship with membership in dairy cooperative societies are access to credit (Gashaw and Kibert, 2018), price of milk, and availability of other services (e.g. fodder seed, subsidized concentrate feed, training, veterinary services etc.) from DCS (Tefera and Wold, 2015).

Results and Discussion

Descriptive statistics of explanatory variables of the model

Table 2 and 3 present the descriptive statistics of the continuous and dummy variables derived from *t*-test and chi square test to compare the differences in the characteristics explaining the DCS membership status. The descriptive statistics display that among the factors the mean income from dairying, per capita consumption of milk, farming experience, milch animal holding, distance to DCS collection centre are found to be statistically different between members and non-members of DCS. Co-operative members have higher income, per capita consumption and employment, large household size and land holding, and more milch cow on average as compared to non-members. According to the results in Table 2, the average annual income of the member group is significantly (p<0.01) higher (INR 259780.7) than the non-member group (INR 152189.3). This is because cooperative members have larger milch animal than non-members, leading to higher milk production and income. Meena and Jain (2012) also recorded a higher significant income for members than non-members households. The per day per capita consumption of milk has been found to be 270.59 grams and 162.03 grams respectively for member against non-member group with p<0.01 significant level. Higher per capita consumption of milk for member farmers shows attainment of higher nutritional status of household members than their counterparts. The mean dairy farming experience of non-members is significantly higher compared to the members. It indicates that some dairy farmers establishing dairy farm newly may prefer to join DCS compared to some experienced farmers with intentions to remain as non-member to source their milk sale in channels other than DCS. There is statistically significant difference (p<0.01) between members and non-members with members having an average of 4.22 milch animals against 3.01 of non-members. In a similar comparison made by Kumar et al. (2013) and Njiru et al. (2015), cooperative farmers have relatively bigger size of herd than the counterparts. It is observed from Table 2 that the mean distance to the DCS collection centre from the non-member households over the members are significantly higher by 0.37 km which is consistent with the findings of Fikadu et al. (2019). The price paid to the DCS members are based on FAT and SNF percent and farmers in the study location always try to maintain milk quality fetching price of INR 38.38/litre, slightly lower than the price fetched by non-members equivalent to INR of 39.04. The statistical difference between members and non-members of the dummy explanatory variables are based on Chi square test and presented in Table 3. Except level of education (for the primary, secondary

Table 1 Description of variables and their expected sign with cooperative membership

Variables	Definition	Measurement	Variable type	Expected sign
Dependent Variable				
MEMBER	Whether the farmer is a member of DCS	1= Member, 0= Otherwise	Dummy	
Independent variables				
GENDR	Gender of the respondent	1= Male, 0= Otherwise	Dummy	+
EDUC	Education level of the respondent	0= Illiterate, 1= Primary, 2= Secondary, 3= HS, 4= Graduate & above	Categorical	+
HHSIZE	No. of family members in a household	No. of family members	Continuous	+/-
FARMEXP	Experience in dairy farming	No. of years	Continuous	+/-
LANDH	Size of landholding of household	Hectare	Continuous	+
MILCH	No of milch animal owned by the household	No. of milch cows measured as SAU	Continuous	+
CREDIT	Whether the respondent has access to credit during five years preceding	1= Yes, 0= No	Dummy	+
OFF-FARMY	Whether household has any off-farm (non-farm) sources of income	1= Yes, 0= No	Dummy	+/-
DISTCOOP	Distance of the cooperative milk collection centre from the farmer house	Kilometers	Continuous	-
PRICE	Price for milk	Rs/kg	Continuous	+
OTHERSERV	Access to other services	1= Yes, 0= No	Dummy	+

and graduate & above group) and access to off-farm income, the other explanatory variables exhibit statistically significant difference in their proportion between the members and non-members. It could also be implied from the previous significant chi square studies (Gashaw and Kibret, 2018) that farmers join cooperatives because of the need to access to institutional and financial services coming from cooperatives.

Impact of Dairy Cooperatives on the wellbeing of sampled households

Cooperatives are conceived as an important vehicle for augmenting production of milk leading to growth in income of the farmers, consumption of self-produced milk and increased use of labour (as potential for growth in employment). In the context of the present study these outcome variables are estimated using multivariate regressions. The main variable of interest is a dummy for whether the household has participated in the cooperatives or not and coefficient estimates are summarized in Table 4. Explanatory variables are checked for multicollinearity and found with absence of any multicollinearity problem (mean VIF 1.14). VIF is a measure of the amount of multicolinearity among the independent variables in a multiple regression model. Detecting multicolinearity is important to measure the correlation between one or more independent variables or inputs and to test certainly how much the combination of independent variables affects the dependent variable within the model. Moreover, results presented in Table 4 show that the F statistic in all the three regression models is statistically significant at 1 percent. Thus the models estimation can be considered as a good fit for the data used in the study. It is seen from the table that controlling for some socio-economic factors such as age and gender of household head, household size, land ownership, access to credit and herd size, the membership of DCS influence positively and significantly the dairy income (by 12.10 percent), consumption of self-produced

Table 2 Descriptive analysis of explanatory variables (continuous) of dairy farmers

Characteristics	Member Mean (Std. Error)	Non-member Mean (Std. Error)	Difference (Std. Error)	*t*-value
Income/year (in Rs.)	259780.7(17762.16)	152189.3(14382.5)	107591.4***(22854.99)	4.7076
Per Capita Consumption (grams/day)	270.592(11.7736)	162.0305(8.6949)	108.5615***(14.6363)	7.4173
Employment (Labour hour/milch SAU/day)	3.0986(0.1827)	3.0826(0.1299)	0.0161(0.2242)	0.0716
Household size (No.)	5.7667(0.2654)	5.42(0.2423)	0.3467(0.3594)	0.9645
Farming experience (Years)	12.4367(0.7750)	15.67(1.0533)	-3.2333**(1.3077)	-2.4725
Land holding (Hectare)	1.0738(0.09486)	0.9099(0.09613)	0.1640(0.1351)	1.2138
Milch animal holding (SAU)	4.22(0.2160)	3.0133(0.1643)	1.2067***(0.2714)	4.4462
Distance to DCS collection centre (km)	2.098(0.1167)	2.4687(0.1437)	-0.3707**(0.1851)	-2.0027
Price of milk (in Rs.)	38.38(0.2607)	39.04(0.3264)	-0.66(0.4177)	-1.5801

Source: Author's estimation based on field survey data;
, * indicate significance level of 5% and 1% respectively

Table 3 Distribution of households by explanatory variables (categorical)

Variables	Character	Member N=150	Non-member N=150	Total N=300	Pearson chi2 test
Gender	Male	137 (91.33)	147 (98)	284	6.6021***
	Female	13 (8.67)	3 (2)	16	
Education					
Illiterate (Base category)					
Primary		22 (14.67)	32 (21.33)	54	2.2584
Secondary		48 (32)	49 (32.67)	97	0.0152
Higher Secondary		47 (31.33)	33 (22)	80	3.3409*
Graduate & Above		31 (20.67)	30 (20)	61	0.0206
Access to credit	Yes	56 (37.33)	8 (5.33)	64	45.7627***
	No	94 (62.67)	142 (94.67)	236	
Access to off-farm income	Yes	49 (32.67)	58 (38.67)	107	1.1767
	No	101 (67.33)	92 (61.33)	193	
Availability of services	Yes	49 (32.67)	16 (10.67)	65	21.3879***
	No	101 (67.33)	134 (89.33)	235	

Source: Author's estimation based on field survey data; Notes: Figures in parentheses indicate percentage to the total.
*, *** are significant at 10 % and 1 % percent level respectively

milk and use of labour on the farming. This finding is in line with the study done by Kumari and Malhotra (2016) where the authors found that the cooperatives have a positive impact on gross income of women dairy farmers. This indicates that taking membership in dairy farming has potential for better livelihood

Table 4 Impact of DCS on the wellbeing of the farmers

Variables	Income	Per capita consumption of milk	Labour hours spent
	Co-efficient(Std. Error)	Co-efficient(Std. Error)	Co-efficient(Std. Error)
Membership of DCS	0.1210***(0.0341)	75.9636***(13.3038)	0.4852**(0.2328)
Age of the HH head	-0.0026*(0.0013)	-0.5337(0.4751)	0.0078(0.0085)
Gender of the HH head	-0.1142(0.0818)	-31.7632(38.1937)	-0.6025(0.3971)
Household size	0.0009(0.0048)	-11.5020***(2.1538)	0.0309(0.0365)
Land owned by the farmer	0.0141(0.0130)	-7.8950(4.8033)	0.0374(0.0771)
Access to credit	0.1633***(0.0365)	47.6752**(19.6054)	-0.0502(0.2513)
Herd size (milch animal equivalent)	0.1104***(0.0101)	15.8885***(3.3741)	-0.4051***(0.0575)
Constant	4.8368***(0.1144)	237.6345***(47.7498)	4.3243***(0.5990)
	R^2= 0.5980	R^2= 0.3247	R^2= 0.2507
	F(7, 292)= 39.21***	F(7, 292)= 14.40***	F(7, 292)= 9.59***
	No of obs. = 300	No of obs. = 300	No of obs. = 300

Source: Author's estimation based on field survey data;
*, **, *** indicate significance level of 10%, 5% and 1% respectively

Table 5 Factors determining dairy cooperative membership

Variables	Co-efficient (Std. Error)	p-value	Marginal effect
Gender	-0.6870 (0.7405)	0.353	-0.1627
Education level Illiterate(base category)			
Primary	-0.0378 (1.0327)	0.971	-0.0094
Secondary	0.6499 (1.039)	0.522	0.1589
HS	0.9110 (1.0247)	0.374	0.2181
Graduate & above	0.4725 (1.0490)	0.652	0.1156
Household size	0.0137 (0.0485)	0.778	0.0034
Farming experience	-0.0289 (0.0132)	0.028**	-0.0072
Land holding	0.0341 (0.1347)	0.800	0.0085
Milch animal holding	0.1877 (0.0709)	0.008***	0.0467
Access to credit	2.2477 (0.4182)	0.000***	0.4573
Access to off-farm income	-0.1751 (0.3103)	0.573	-0.0436
Distance to DCS collection centre	-0.3271 (0.1055)	0.002***	-0.0815
Price of milk	-0.1047 (0.0430)	0.015**	-0.0261
Availability of services	1.1765 (0.3687)	0.001***	0.2728
Constant	3.9740 (2.0049)	0.047**	
Pseudo R square	0.2376		
Wald Chi square	72.22***		
Log pseudo likelihood	-158.53229		

Source: Author's estimation based on field survey data;
, * indicate significance level of 5% and 1% respectively

and nutritional support among the member farmers compared to the farmers without membership of DCS.

Factors determining membership of DCS

Table 5 presents the logistic regression results for the factors that influence farmers' membership in dairy cooperatives. It can be observed from the table that the model is statistically significant and model is a good fit as explained by Wald chi square value of 72.22 (p<0.01) and Pseudo R square of 0.2376 indicating that 23.76 percent of the variations in probabilities of taking cooperative membership was explained by the covariates defined in the logistic model. Out of the explanatory variables included in the model, six variables were found to be statistically significant to influence farmers' membership in dairy cooperatives. Among these variables; milch animal holding, access to credit and availability of other services rendered by DCS positively influence the membership decision. On the other hand, dairy farming experience, distance to the DCS collection centre and price of milk negatively influence the decision of the farmers to join cooperative societies.

Farmers' experience in dairy farming, also considered as a proxy for age of the farmers influence negatively the DCS membership. It indicates that farmers starting dairy business newly show more interests to join DCS compared to the farmers who are involved in dairying for a longer time. The value of marginal effect implies that when experience increases by one more year, the probability of membership in dairy cooperatives decreases by 0.72 percent. Farmers with relatively higher herd sizes are more likely to join DCS as explained by milch animal holding having positive and statistically significant (p<0.01) effect on DCS membership. This indicates that addition of one more SAU of milch cow to the farmers' herd increases the probability of a farmer becoming DCS member by 4.67 percent. The finding is supported by other studies such as Bardhan and Sharma (2012). The positive and significant (p<0.01) relation between the access to credit and cooperative membership indicates that having access to credit leads to 45.73 percent higher probability of becoming member. This result coincides with the findings of Nirju et al. (2015). There are instances of accessing small credits from DCS which also attract the farmers towards joining DCS. Gashaw and Kibret (2018) shows that this source of credit is a reliable one compared to that of formal lending institutions and traders associated with collateral agreements. It is evident from Table 5 that larger the distance from the farmers' house to cooperative collection centre, the lower the probability of becoming member of DCS. This is explained by the fact that farmers who are close to the cooperative office will also have more knowledge about the cooperative benefits and will also have lower transaction costs. The result is in line with the findings of Fikadu et al. (2019), Chagwiza et al. (2016) and Ahmed and Mesfin (2017). The association between price fetched by selling milk and probability of becoming members of DCS indicates that increase in the milk price by INR 1.00 would decrease the likelihood of farmers' membership to DCS by 2.61 percent. In a study carried out in the context of Assam, Bayan (2018) showed that the price received by cooperative members are already low, for which farmers may not be inclined to join DCS leading to negative relationship between membership and price received for the milk sold. In addition to this, farmers are also discouraged by the duration of payment as explained by them that they have to wait several days for getting paid, but they are in urgent cash needs for maintaining day to day expenses of cattle feed. Availability of other services (e.g., access to concentrate feed at subsidized rate, fodder seed, veterinary services, training etc.) from DCS positively and significantly (P<0.01) relates to farmers' membership. This result is in line with some other studies such as Debeb and Haile (2016), Tefera and Wold (2015).

Conclusions

The study has shown a greater role of DCS on increasing dairy income, higher consumption of self-produced milk and rise in employment through higher use of labour. This indicates the need for emphasis on the diffusion of cooperative forms of dairying in many districts of Assam due to its livelihood and nutritional impact on the smallholder farmer's economy. The study suggests generating certain instrumental factors such as access to credit through dairy cooperatives to meet the financial viability, establishing more collection centre and improving market infrastructure to enable members to meet their needs. Moreover, if cooperatives are continued to be adopted as medium of offering services to dairy farmers like fodder seed, subsidized concentrate feed, veterinary services etc., this will motivate farmers to take its membership. Access to these extension services will ensure improved dairying which will scale up milk production and income of the farmers. Given the importance of urgent cash needs for daily transactions, it is strongly recommended that cooperatives pay more attention to it in case of expediting membership. Overall, to increase farmer's membership the study recommends policies to take up more awareness programmes and trainings from time to time among the younger farmers on the beneficial effects of participating in cooperative system of dairying. A proper policy review should be necessary action with all limitations and functional trade-offs to ensure enough incentives which would favour membership growth and livelihood of farmers beyond this study.

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(Footnotes)

[1] SAU (standard animal unit): 1 Crossbred= 1.40 SAU; 1 Indigenous= 1.00 SAU

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SHORT COMMUNICATION

Risk association of metabolites and immune response mediator indicators with the occurrence of retained placenta in Murrah buffaloes (*Bubalus bubalis*)

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Abstract: This study was designed to investigate the risk association of metabolites and immune response mediator indicators with the occurrence of retained placenta (RP) in Murrah buffaloes (*Bubalus bubalis*). A total of six (n=6) healthy pregnant Murrah buffaloes and four (n=4) buffaloes suffering from RP were selected from the ICAR-NDRI cattle herd. Blood samples were collected from each healthy buffalo on days -56,-48,-42,-35, -28, -21, -14, -7, 0, +7, +14, +21, +28, +35, +35, +42, +48, +56 relative to calving. Blood samples were also collected from the buffaloes on the day of diagnosis of the RP as well as on an alternate day. The result revealed that plasma β-Hydroxybutyric acid (β-HBA) and nitric oxide (NO) levels were significantly ($P< 0.05$) higher in buffaloes suffering from RP compared to normal parturient buffaloes. The level of IgG was significantly ($P<0.05$) lowered in buffaloes suffering from RP (8.84±0.85 mg/mL) compared to normal parturient buffaloes (17.41±1.68 mg/mL). However, plasma Non-esterified fatty acids (NEFA), glucose, Blood Urea Nitrogen (BUN), calcium, Interleukin 6 (IL-6), and Total Antioxidant Activities (TAA) levels were non-significant differences between buffaloes suffering from RP and normal parturient buffaloes. A binary logistic regression assay revealed the positive association of postpartum β-HBA with RP (Odds ratio= 1.85). Overall results suggest that plasma β-HBA, IgG, and NO can be used as screening biomarkers during the transition period for the risk assessment of RP in buffaloes.

Keywords: Buffaloes, β-HBA, IgG, Nitric oxide, Retained placenta

The retained placenta is a common multifactorial postpartum reproductive disease manifesting as failure to expel fetal membranes within 12 hours of calving. RP causes huge financial loss to the dairy industry due to increasing the risk of postpartum infections, infertility, and reduced milk yield and quality (Moretti et al. 2015; Mahnani et al. 2020; Li et al. 2021). The etiology and pathogenesis of RP has been investigated extensively by many researchers to explore early diagnosis in dairy cattle. Many studies have also confirmed that changes in blood metabolites, cytokines, inflammatory factors, immune factors, and hormones are associated with the pathogenesis of RP (Esposito et al. 2014; Moretti et al. 2015; Lu et al. 2020, Mili and Pandita 2021; Li et al. 2021). The changes in metabolism, immune response mediators, and hormonal variables during the transition period are part of homeorhesis in buffaloes (Mili et al. 2014; Mili et al. 2015a; Mili et al. 2015b). The detection of blood biochemical indicators are the most common method for predicting and screening diseases. Hence, the present study aimed to find the risk association of the key metabolites and immune response mediators with the occurrence of RP in buffaloes.

The present experiment was conducted between September 2011 till May 2012 at ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana. The institute is located at an altitude of 250 m above mean sea level, latitude, and longitude positions 29°42N and 79°54E, respectively. The maximum ambient temperature in summer goes up to 45°C, and the minimum temperature in winter comes down to 0°C with a diurnal variation in the order of 15-20°C. The average annual rainfall is 700 mm from early July to mid-September.

A total of six (n=6) numbers of healthy pregnant Murrah buffaloes and retained placenta (n=4) were selected from the institute cattle herd. The buffaloes that did not shed the fetal membrane within 12 hours of parturition were considered cases of RP. All these

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buffaloes were maintained under general managemental practices as followed at the institute.

A blood sample (15ml) was drawn in sterile heparinized vacutainer tubes by jugular venipuncture from each healthy buffalo on days -56, -49,-42,-35,-28, -21, -14, -7, 0, +7, +14, +21, +28, +35,+42, +49, +56 relative to calving. Also, blood sample was collected on the day of diagnosis of the RP and as well as on an alternate day. The heparinized samples were centrifuged at 3000 rpm for 15 minutes, plasma aliquot, and stored at -20°C for further analysis.

NEFA levels were quantified as per the copper soap solvent extraction method modified by Shipe et al. (1980). β-HBA was estimated by "β-Hydroxybutyrate (Ketone Body) Assay Kit -1" of Cayman Chemical Company, Ann Arbor USA as per manufacturer instruction. Glucose, BUN, albumin, and calcium levels were quantified using GOD-POD kits obtained from Span Diagnostics Ltd, respectively as per manufacture instructions.

IgG, IL-6, and TAA levels were estimated by using "Bovine IgG ELISA kit" procured from Koma Biotech Inc., Gangseo-gu Seoul, Korea, bovine interleukin-6 ELISA Kit" procured from Cusabiotech and Antioxidant assay kit purchased from Cayman Chemical Company, Ann Arbor USA respectively as per the instructions provided with the assay kits. The NO levels were quantified using a modified Griess reaction as described by Shoker et al. (1997).

All the values were expressed as mean ± standard error (SEM). The data for healthy buffaloes were analyzed by one-way analysis of variance using a graph prism version 5 to quantify postpartum variations for peripheral levels of NEFA, β-HBA, glucose, calcium, BUN, albumin, IgG, IL-6, NO, and TAA. Since postpartum variations were not statistically significant between days (Already published by Mili et al. 2014 & Mili et al. 2015a), the data for each parameter was clubbed. This served as the reference value for healthy buffaloes for binary logistic regression assay to evaluate the risk association of metabolic variables and immune response mediator indicators with the occurrence of RP in SAS software (7.0 versions). Also, the unpaired student "t" test using graph prism version 5 was applied to compare the data of normally calved buffaloes (day-0) and RP.

The changes in plasma β-HBA, NEFA, glucose, BUN, albumin and calcium levels in buffaloes suffering from RP compared to normal parturient buffaloes is presented in Table 1. Plasma NEFA level was non-significantly very high in buffaloes suffering from RP (418.49±34.00 µmol/L) compared to normal parturient buffaloes (406.69±20.60 µmol/L) with an odds ratio of 0.002. The odd ratio indicated a negative association of plasma NEFA with the occurrences of RP in buffaloes. High concentrations of serum NEFAs have been associated with an increased incidence of periparturient diseases (retained fetal membranes, ketosis, and mastitis), displacement of the abomasum and immune-suppression in dairy cattle (Leblanc et al. 2005). Plasma β-HBA level was significantly high (P<0.05) in RP (591.20±32.74 µmol/L) compared to normal parturient buffaloes (301.61±32.74) with an odds ratio of 1.85. The odds ratio indicated a positive association of plasma β-HBA with the occurrences of RP in buffaloes. Our results were in agreement with previous studies (Seifi et al. 2007; Lazlo et al. 2009; Quiroz-Rocha et al. 2009). Seifi et al. (2007) reported greater concentrations of plasma NEFA and β-HBA in cows with RP. Elevated NEFA and ketone bodies are metabolic indicators of increased risk for RP in cows (Lazlo et al. 2009; Quiroz-Rocha et al. 2009).

Plasma glucose level was no significant association (47.61±2.94 mg/dL in RP buffaloes vs- 47.81±2.32mg/dL normally calving buffaloes) with the occurrence of RP (odds ratio= 0.002) in buffaloes. In contrast to our results, Mandali et al. (2002) and

Table 1 Plasma metabolite concentration in buffaloes exhibiting retained placenta

Parameters	Day 0(Normally calved)	RP(After 12 h of parturition)
NEFA (µmol/L)	406.69±20.60	418.49±34.00
β-HBA (µmol/L)	301.61±32.74^{A}	591.20±32.74^{B}
Glucose (mg/dL)	47.81±2.32	47.61±2.94
BUN (mg/dL)	21.24±2.17	13.72±0.82
Calcium (mg/mL)	6.24±0.24	5.40±0.58

Bearing superscripts AB in rows differ significantly (P < 0.05) from each other

Table 2 Plasma immune response mediators in buffaloes exhibiting retained placenta

Para-meters	Day 0(Normally calved)	RP(After 12h of parturition)
IgG (mg/mL)	17.41±1.68^{A}	8.84±0.85^{A}
IL-6 (pg/mL)	25.00±4.33	19.72±1.29
TAA (mmo/L)	1.88±0.16	1.15 ±0.09
Nitric oxide (µmol/L)	50.88 ±1.41^{A}	60.97 ±2.96^{B}

Bearing superscripts AB in rows differ significantly (P < 0.05) from each other

Pandey et al. (2009) reported significantly lower (P<0.05) glucose levels in buffaloes with RP than in normal parturient buffaloes.

The plasma calcium level was not significantly lower in buffaloes suffering from RP (5.40±0.58 mg/mL) compared to normal parturient buffaloes (6.24±0.24 mg/mL). This result was in agreement with the earlier reports (Abo El Maaty et al. 2021). In contrast to our results, various reports revealed that inadequate calcium concentrations are the predisposed risk factor for occurrences of RP in cattle and buffalo (Hashem and Amer 2008; Pandey et al. 2009). Mohanty et al. (1994) suggested that probably less availability of glucose and calcium to the uterine tissues results in atony of the uterus, with decreased contraction and hence the retention of fetal membranes. Calcium deficiency could act as a predisposing factor for uterine inertia leading to dystocia, RP, and metritis (Mohanty et al. 1994).

Plasma BUN level was not significantly lower in buffaloes suffering from RP (13.72±0.82 mg/ dL) compared to normal parturient ones (21.24±2.17mg/dL) with an odds ratio of 0.002. Our result was in agreement with previous studies on dairy cows (Lu et al. 2020). However, they predicted that serum BUN levels above 10.25 mg/ dL on day 7 relative to parturition are the predisposing risk factors for occurrences of RP in cows (Lu et al. 2020).

The changes in immune response mediator indicators IgG, IL-6, TAA, and NO levels in buffaloes suffering from RP compared to normal parturient buffaloes is presented in Table 2. The level of IgG was significantly (P<0.05) lower in buffaloes suffering from RP (8.84±0.85 mg/ mL) compared to normal parturient buffaloes (17.41±1.68 mg/mL). However, the TAA level was non significantly low in buffaloes suffering from RP (1.15 ±0.09 mmol/L) compared to normal parturient buffaloes (1.88±0.16 mmol/L). Mili et al. (2015a) reported a gradual drop in IgG and TAA levels in buffaloes from day 56 before parturition to the lowest levels on the day of calving. Hence, reduced availability of antioxidant defenses, gradual changes of humoral immune response (IgG and IgM) to a cell-mediated immune response with progressive oxidation of the cell membrane during the transition period may contribute to periparturient disorders including RP in dairy cows (Miller et al. 1993; Gitto et al. 2002).

Plasma IL-6 levels were also non significantly low in buffaloes suffering from RP (19.72±1.29 pg/mL) compared to normal parturient buffaloes (25.00±4.33 pg/mL). Our result was in agreement with the earlier reports (Dervishi *et al.* 2016). They revealed that the overall serum IL-6 concentration was not different between RP and normal parturient cows. But, serum IL-6 concentration in RP cows at 8 weeks before parturition was significantly higher than normal parturient cows. IL-6 plays a significant role during the transition from innate to adaptive immunity. Elevated levels of both IL-1 and IL-6 suggested the presence of an inflammatory insult. Hence, deviations from the normal reference ranges of both IL-1 and IL-6 during the transition period are the risk indicators of the development of RP in dairy cows (Dervishi *et al.* 2016).

The plasma nitric oxide levels were significantly (P<0.05) high in buffaloes suffering from RP (60.88±2.96 µmol/L) compared to normal parturient buffaloes (50.88 ±1.41 µmol/L). This result was in agreement with the earlier reports (Abo El Maaty *et al.* 2021). They reported significantly higher NO in cows suffering from RP (33.49±5.80µmol/L) compared to normal parturient cows (26.83±1.81µmol/ L).

Conclusion

It was concluded that alteration of plasma metabolites and humoral immune response coupled with a low antioxidant defense system as monitored by β-HBA, IgG, and NO levels during the transition period might be the risk indicators with the occurrence of retained placenta in buffaloes. However, large-scale studies are required to determine the crucial threshold levels of these attributes to trace the onset/ early diagnosis of RP in buffaloes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SHORT COMMUNICATION

An investigation on morphometric measurements and adaptability of Marathwadi buffaloes in the native breeding tract

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Abstract: The present study was carried out on 204 Marathwadi buffaloes, 154 from Livestock Farm Complex (LFC), College of Veterinary and Animal Sciences (COVAS), Udgir, Dist. Latur and 50 from villages of Udgir tahsil to analyse their growth and adaptability profile. Marathwadi buffaloes were divided (age wise) into three groups (0-1 year, 1-3 year and 3 year and above). Different biometrical and physiological parameters were recorded during five consecutive years to study the growth profile and adaptability of Marathwadi buffaloes. No significant difference was observed in morphological parameters (body length, chest girth, height at withers, body weight and body surface area) between buffaloes reared in LFC, COVAS, Udgir and under field conditions of adjoining villages in Udgir Tahsil. Body growth pattern was proportionate according to age in both the groups. However, growth parameters were comparatively lower in buffaloes under village conditions than buffaloes maintained at LFC, COVAS, Udgir. Benezra's Coefficient of Adaptability (BCA) was used to assess the adaptability of these buffaloes and no significant difference was observed among the both group of buffaloes across all age groups. However, as per Iberia Heat Tolerance Coefficient (IHTC), adaptability values were nearby 100 in all groups and there was no significant difference was observed between these two groups. It is inferred that, growth pattern was proportionate with age in Marathwadi buffaloes which were reared under organised farm as compared to the animals reared under field condition.

Keywords: Adaptability, Growth profile, Marathwadi buffaloes

Adaptation is often at the expense of performance, and survivability is often better in "low" performance animals because their input needs (especially feed) and internal heat production are not as great (Gaughan and Smith, 2017). Marathwadi buffalo is an indigenous breed of buffalo which is distributed in Marathwada region viz. Nanded, Parbhani, Dharashiv and Latur districts of Maharashtra state. This buffalo breed is well survived in this region and thrive mainly on agricultural crop residues feeding and to rear this buffalo require less external inputs as reported earlier by Bande et al. (2018) and therefore, farmers of this region mostly prefer Marathwadi buffalo as milch buffalo. Light to medium body built, predominantly black colour, long flat horns, 3.5–4.0 litter daily average milk production and regular breeder are some productive and reproductive characteristics of Marathwadi breed of buffalo (Joshi, 2010). Farm animals body growth and production performance are generally dependents on their genetic constituents, nutrition pattern, health status and routine management practices. Periodic assessment of growth profile is essential to judge physiological, metabolic and health status of farm animals for modification in management practices for slow growing animals. Farm animal's biometrical measurements and physiological profiles are different in different breeds of similar species and therefore, standard baseline data is required to assess their productivity in different geographical locations. Different breeds of indigenous buffalo are adapted in their origin with optimum productivity however; body growth profile and adaptability studies are most important criterion to assess nutritional, reproductive and productive status in their breeding tract. Best of our knowledge, very scattered and limited data is available on Marathwadi buffalo growth profile and adaptability studies and hence, it was planned to analysed growth profile and adaptability of Marathwadi buffalo.

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Table 1 Age-wise comparison of morphometric measurements and adaptability parameter of Marathwadi buffaloes

Parameters	Buffaloes groups	Age (years)			Student's Unpaired t-test
		0-1	1-3	3 and above	
Body length (cm)	COVAS, Udgir	76.4±1.0	95.6±2.2	140.66±3.3	
	Nearby Villages	72.0±1.0	93.9±2.6	124.9±1.9	NS
Chest girth (cm)	COVAS, Udgir	96±1.4	127±3.3	179.4±1.9	
	Nearby Villages	84.0±1.6	117.5±4.0	171.6±1.6	NS
Height at withers (cm)	COVAS, Udgir	84.4±1.6	98.6±2.1	135.2±2.2	
	Nearby Villages	76.0±1.1	97.0±3.6	124.1±1.3	NS
Body weight (kg)	COVAS, Udgir	63.5±2.7	146.0±4.7	392.7±4.4	
	Nearby Villages	59.4±1.1	142.9±2.4	314.0±2.8	NS
Body surface area (m^2)	COVAS, Udgir	1.4±0.0	2.4±0.1	4.3±0.0	
	Nearby Villages	1.4±0.0	2.4±0.0	3.8±0.0	NS
BCA values	COVAS, Udgir	1.3±0.0	1.2±0.0	1.1±0.0	
	Nearby Villages	1.6±0.0	1.5±0.0	1.4±0.0	NS
IHTC values	COVAS, Udgir	103.1±0.7	100.0±0.7	96.6±0.9	
	Nearby Villages	111.0±0.9	106.4±0.7	103.8±0.5	NS

(Note: NS= Non-Significant different between two groups)

The present study was carried out on a total of 204 Marathwadi buffaloes, 154 from Livestock Farm Complex, College of Veterinary and Animal Sciences Udgir, Dist. Latur (Maharashtra) and 50 Marathwadi buffaloes from nearby villages were selected for this study. Selected buffaloes of both the groups were divided accordingly age wise into 0-1 year, 1-3 year and 3 year and above and study was conducted during five consecutive years which was started in year 2016-17 and completed in year 2020-21. Biometrical measurements such as body length (cm), chest girth (cm), height at withers (cm), body weight (kg) and body surface area (m^2) were estimated by routine methods and analysed growth profile of buffaloes. Buffalo body surface area (BCA) was calculated by Brody S. (1945) equation and physiological responses (respiration rate, pulse rate and rectal temperature) of each buffalo were recorded thrice in a year during study. The average values of physiological responses were used to determine adaptability by two methods i.e. Benezra's Coefficient of Adaptability (BCA) (Benezra, 1954) and Iberia Heat Tolerance Coefficient (IHTC) (Rhoad,1944). Data was statistically analysed by using t test (Snedecor and Cochran, 1994) and mean values and standard errors were calculated and presented in tables.

Body length, chest girth, height at withers, body weight and body surface area of Marathwadi buffaloes were proportionate with increasing age in both the groups of buffaloes. No significant difference was observed in morphological parameters between LFC, COVAS, Udgir and village reared buffaloes. However, it was noticed that the values of different parameters of village reared buffaloes were numerically lower than the buffaloes reared at LFC, COVAS,Udgir (Table 1).Growth rate of growing Marathwadi buffaloes (0-3 years) was slower as compared with Murrah buffaloes (Mishra et al. 2015). However, with the increasing age of Marathwadi buffalo (3 year and above), body length, chest girth, height at withers, body weight and body surface area were proportionately increased in both the groups which is in accordance with the findings of Joshi (2010). Our findings indicated that, body growthprofile of Marathwadi buffaloes which were maintained at LFC, COVAS, Udgir were better than buffaloes reared by farmers in nearby villages. Therefore, additional managemental practices such as proper shelter, feeding and watering should be followed during summer season by farmers for achieving standard growth and optimum production from Marathwadi buffaloes in their breeding tract. Adaptability coefficient of farm animals were estimated by recording physiological responses (respiration rate and rectal temperature) and calculated values as 2 and 100 are considered as ideal value for BCA (Benezra, 1954) and IHTC method (Rhoad, 1944), respectively. As per the BCA, Marathwadi buffaloes of all age groups that maintained by farmers and LFC, COVAS, Udgir were adapted (Table 1) where, BCA values were less than 2 however, slight numerical difference was observed in BCA values between LFC,COVAS, Udgir and villages reared buffaloes. It was indicated that there was better managemental practices followed for Marathwadi buffaloes which were maintained at LFC, COVAS, Udgir compared with buffaloes reared by farmers in nearby villages. Adaptability coefficient values calculated by IHTC were nearer to 100 in both buffalo groups (Table 1) with little difference in between groups and it was observed that, buffalo which were maintained at LFC, COVAS, Udgir were adapted well as compared with village reared buffalo.

The Iberia Heat Tolerance Coefficient (IHTC)values for village reared buffaloes were lightly more than 100 (Table 1) which showed that, these buffaloes were comparatively less adapted as compared with buffaloes which were reared at LFC, COVAS, Udgir. BCA method is based on respiration rate and rectal temperature and whenever, there is increased in surrounding environmental temperature, animals respiration rate is being

increased and heat quickly dissipated from the animals. Therefore, adaptability coefficient that determined by BCA method is might be less than 2 in all buffalo groups. However, adaptability values calculated by IHTC method were nearer to 100 in all groups and there was no significant difference was observed between village and LFC, COVAS, Udgir reared buffalo groups, Our findings are in accordance with Vaidya et al. (2022) they observed IHTC in 3 years and above age groups in of Marathwadi buffaloes which showed better adaptability compared to the age groups 0-1 year and 1-3 years, It indicates that the adaptability of the Marathwadi buffalo to the harsh climate of Marathwada increased with an increased in the age. BCA and IHTC are heat tolerance indices for farm animals that based on physiological indices (respiration rate and rectal temperature) and values of these physiological indices increase with increase in environmental temperature and adaptability values may be slightly alter. Therefore, proper thermal protective measures are required for Marathwadi buffaloes (Kalyankar et al. 2004) which are reared by farmers in nearby villages to enhance their reproductive and productive performances in their breeding tract.

Conclusions

The body growth pattern of Marathwadi buffaloes reared under field and organised farm were proportionate with age. However, for better adaptability, thermo-protective care should be taken by farmers during adverse climatic conditions to sustain productivity. Marathwadi buffaloes were well adapted in their native breeding tract as per Benezra's Coefficient of Adaptability (BCA) and Iberia Heat Tolerance Coefficient (IHTC) with no significant difference between buffaloes reared under field condition and organised farm.

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SHORT COMMUNICATION

A simple and cost effective method to detect adulteration in ghee with vegetable oils through microscopic examination of sterols

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Abstract: Plant sterols together called as phytosterols, and animal sterols mainly cholesterol differ from each other in their crystal shapes, apart from other differences in terms of chemical structure, melting points, etc. When viewed under the microscope, after their isolation from the samples in purified state, phytosterols crystals appear hexagonal in shape while those of cholesterol acquire parallelogram shape. But the mixture of phytosterols and cholesterol shows the crystal structure with re-entry angle (Swallow's tail).On the basis of this characteristic crystal shape, detection of vegetable oils in milk fat up to a level of 15 percent could be confirmed with this simple cost effective method . However, this approach cannot be applied for the detection of body fats in milk fat because of the existence of common sterol (cholesterol) in them.

Keywords: Ghee adulteration, Microscopic examination, Sterols, Vegetable oils

Lipids represent one of the most important constituents of milk and milk products. In India, milk fat is mostly consumed in the form of ghee (clarified butterfat). Due to its short supply and more demand, expensiveness (costing 3 to 4 times as much as edible vegetable oils) and variable chemical composition, ghee falls prey to adulteration by the unscrupulous traders in the market. The commonly used adulterants include vegetable oils and fats, animal body fats, mineral oils, etc. Detection of foreign fats in milk fat is a very complex phenomenon, almost comparable with the detection of Pacific water in a sample of Atlantic water. No single test is available to detect all types of adulterants in ghee. Several methods (Kumar et al. 2002 , Boghra et al. 1981, 2004, Molkentin , 2007, Gutierrez et al. 2009, Amrutha Kala et al. 2016 , Rani et al. 2016, Aparnathi et al. 2019, Kumar et al. 2019, Shinde et al. 2020) have been developed in the past to detect the adulteration in ghee. These methods were mostly based on chemical parameters like fatty acid composition and the physico-chemical constants. But few attempts have been made to detect the adulteration on the basis of minor components such as sterols, cis-trans isomers, poly unsaturated fatty acids(PUFA) etc. (Molkentin, 2007, Gutierrez et al. 2009 , Rani et al. 2016, Zychowski et al. 2016, Aparnathi et al. 2019, Kumar et al. 2019, Nurseitova et al. 2019, Khorsandmanesh et al. 2020, Shinde et al. 2020). All these methods require the use of sophisticated instruments like GC-MS, HPLC, which are very costly and require lengthy preparatory steps for the analysis of the fatty acids and sterols as markers for detecting the adulteration with vegetable oils. Sterol profile determination was found more efficient than fatty acid analysis (Rachna and Nath, 2008, Zychowski et al. 2016, Nurseitova et al. 2019,2021, Khorsandmanesh et al. 2020, Shinde et al. 2020).

Sterols represent the major constituent of the unsaponifiable matter and range from 0.24 to 0.50 percent in butter fat, 0.03 to 0.14 percent in body fats and 0.03 to 0.50 percent in vegetable oils. Plants and animal fats have different types of sterols. Animal fats have cholesterol as the characteristic sterol while plant fats have phytosterols, which include β-sitosterol, stigmasterol, campesterol, brassicasterol etc. (Bailey, 2005, Christie, 2014, De, 2019, McSweeney et al. 2020).

On the basis of microscopic structure of sterols, plant fats can be differentiated from milk fat, while body fats cannot be distinguished from milk fat because both body fats and milk fat have cholesterol as the common sterol. Phytosterols and cholesterol differ from one another in a number of properties like crystal shape, Resolution factor (R_f) value, melting point, etc (Gurr et al. 2008, Fox, 2012; Christie, 2014, De , 2019, McSweeney et al. 2020). Therefore, in the present study, a simple method of

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microscopic examination of characteristic structure of sterols has been used as a criteria for checking purity of milk fat suspected with the presence of vegetable oils .

Milk used for the preparation of ghee samples was collected from the Institute's cattle yard. Cow milk was a mixture of the milk obtained from the herd of Karan Swiss, Karan Fries, Sahiwal and Tharparkar breeds. Buffalo milk used was also the herd milk from Murrah breed only. Cows and buffaloes were maintained under identical conditions of feeding and management. Soon after the collection of milk, it was warmed to 40^0C and separated into cream, using mechanical cream separator. The cream was pasteurized at 77^0C for 5 minutes, cooled to room temperature and then kept in a refrigerator (5 to 10^0C) for 3 to 5 hours for ageing. Butter was prepared under standard conditions (9^0C in summer and 13^0C in winter) by churning the cream using hand churn.

The vegetable oil was added to pure ghee (buffalo as well as cow) at the butter stage on the basis of its fat content at 5, 10 and 15 % levels. The butter samples admixed with the adulterants were clarified on direct flame in a stainless steel vessel under continuous stirring at temperature of 120^0C/flash and finally filtered through Whatman No. 4 filter paper. Simultaneously, pure ghee sample (control) was also prepared under similar conditions from the same lot of butter.

Detection of vegetable oils in ghee samples was carried out according to standard methods (IS:3508, 1966 and IDF, 1965). The method involves saponification of fat sample followed by precipitation of sterols with alcoholic digitonine solution. Sterol digitonides were then acetylated using acetic anhydride followed by saponification to obtain the crystal form of sterols to be viewed microscopically. The method, in brief, was as follows:

Accurately, 15 g of the fat samples were weighed in a 250 ml conical flask and saponified after adding 10 ml of the potassium hydroxide solution (66.7%, w/v), 20 ml of ethanol (95 to 96%, v/v) and 2 to 3 glass beads. Then 60 ml of the distilled water and 180 ml of ethanol (95 to 96%, v/v) were added followed by the addition of 30 ml of the alcoholic digitonine solution (1%), shaking and cooling. The flask was placed in a refrigerator at about 5°C for about 12 hours followed by filtration through Whatman No.1 filter paper. The precipitates of sterol digitonide thus obtained were washed with water at about 5°C until the filtrate stopped foaming, followed by washing once with 25 to 50 ml ethanol (95 to 96%, v/v) and then finally with 25 to 50 ml diethyl ether, and dried in an oven at 102 ± 2°C for 10 to 15 min.

To about 100 mg of the dried sterol digitonide precipitate obtained, 1 ml of acetic anhydride was added and heated in a glycerol bath maintained at 145°C until the precipitate had dissolved. Heating was continued for 2 minutes, followed by cooling to about 80°C. Then 4 ml of ethanol (95 to 96%, v/v) was added, mixed, heated slightly and filtered through a small medium speed filter paper impregnated with ethanol. The filtrate obtained was heated and brought to gentle boiling. While still boiling, drop-by-drop of 1 to 1.5 ml of distilled water was added carefully until the steryl acetate was just about to precipitate but still remained in the solution.

A few drops of ethanol (95 to 96%, v/v) were added to dissolve again any precipitated sterol acetate and allowed to cool in the air for 2 hours and finally in ice water for 30 minutes. The crystals of steryl acetate formed on cooling were filtered on a fast speed filter paper (Whatman No. 4) and rinsed with 1 ml of ethanol (80%, v/v). The crystals thus obtained were redissolved by heating in 1 ml of ethanol (95 to 96%, v/v) and subsequently allowed to cool first in air for 15 minutes and then in ice water for 5 minutes. The fresh crop of crystallized sterol acetate was again filtered as described above. Again it was redissolved, crystallized and filtered to get the third, occasionally the fourth or fifth recrystallization. The crystal cake was dried on the filter paper first in the air (about 30°C) and then at 102°C ± 2°C in drying oven for 10 to 15 minutes.

About 10 mg of the sterol acetates purified as above were taken in a test tube followed by the addition of 1 ml ethanol (95 to 96%, v/v) and 1 or 2 drops of potassium hydroxide solution. The tube was heated on a boiling water bath until the boiling began and steryl acetate had dissolved. This solution was then transferred to a 125 ml separating funnel with the help of 10 ml distilled water. The sterols were extracted with 25 ml of diethyl ether. The ether layer was washed with 3 to 5 ml portions of distilled water and evaporated to dryness. The residue was dissolved in 10 ml of ethanol (80%, v/v). Then drop of the clear solution was placed on a microscope cover slip, waited until the crystallization started at the periphery of the drop, then the cover slip was inverted and laid on a microscope slide and examined under microscope at about 200 X linear magnification.

If the sterol crystals are found to have only the form of a parallelogram with an obtuse angle (100°), which is characteristic for cholesterol, the fat sample is considered to be free from vegetable fat. However, if some of the sterol crystals show the elongated hexagonal form with an apical angle (108°), which is characteristic for phytosterols or if some of the crystals have a re-entry angle (Swallow's tail), which is characteristic for mixtures of cholesterol and phytosterol, the fat sample is considered to contain vegetable oils /fat.

The sterol crystals, obtained by saponification of fat samples followed by alcoholic digitonization, acetylation and subsequent saponification, were viewed under microscope for their characteristic shapes.

Results on the microscopic examination of the sterol crystals of standard cholesterol, cholesterol isolated from pure buffalo ghee9Buffalo and cow) , standard phytosterol (stigmasterol),

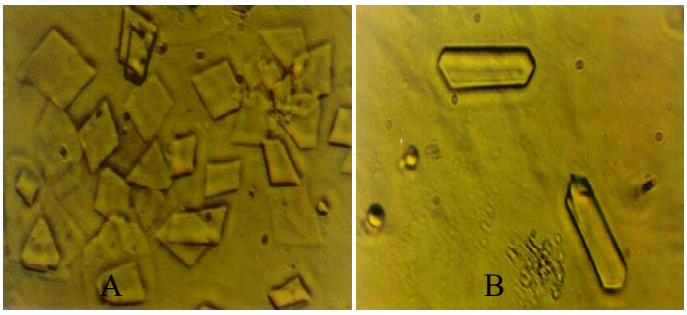

Fig. 1 Micrograph showing the sterol crystals of pure buffalo ghee (A) and groundnut oil (B)

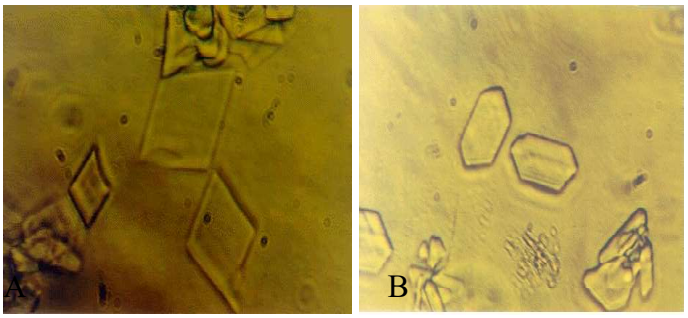

Fig. 2 Micrograph showing the crystals of standard cholesterol (A) and standard phytosterol (B)

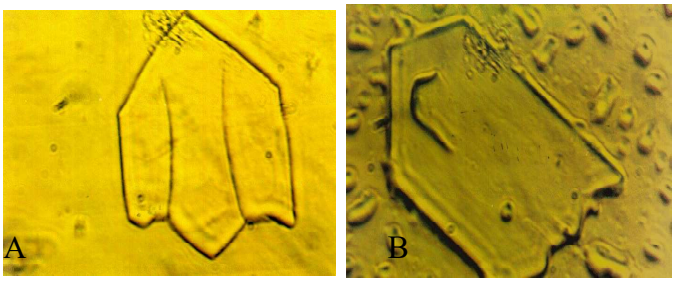

Fig. 3 Micrograph showing the sterol crystals of ghee adulterated with 15 percent groundnut oil (A) and a mixture of standard cholesterol and standard phytosterol in equal proportion (B)

phytosterols of pure groundnut oil, mixture of standards of cholesterol and stigmasterol, and sterols of buffalo ghee adulterated with groundnut oil at 15 percent level are depicted in Figs. 1to 3

Crystals of pure standard cholesterol and those isolated from pure buffalo ghee showed the characteristic parallelogram structures, while those of standard phytosterol and groundnut oil showed the characteristic hexagonal structures. On the other hand, crystals of mixture of standard cholesterol and phytosterol, and those of adulterated ghee samples showed a characteristic crystal structure with re-entry angle (Swallow's tail).

In the present study, the ghee samples adulterated with vegetable oils up to 10 percent level failed to show the expected type of crystal structure with re-entry angle (Swallow's tail), whereas the ghee sample adulterated with vegetable oil at 15 percent level exhibited these very clearly. The results obtained in the present study are supported by findings of Den Herder (1955) also who studied the detection of adulteration of butter with foreign fats on the basis of sterol structure , and indicated that if the sterol crystals show only the parallelogram, which is characteristic for cholesterol, the milk fat is considered to be free from vegetable oils and fats. Whereas, if the sterol crystals show the shape of hexagonal form, which is characteristic for phytosterols or if some of the sterol crystals have a re-entry angle (Swallow's tail) which is a characteristic for mixtures of cholesterol and phytosterols, the milk fat is considered to be adulterated with vegetable oils and fats. However, he further observed that crystals showing re-entry angle (Swallow's tail) are observed only when the percentage of phytosterol in a mixture of cholesterol and phytosterol exceeds 8 percent, which corroborated our findings. Therefore, adulteration of ghee samples with 15 percent groundnut oil could be confirmed by using this simple parameter.

Conclusion

In the present study, microscopic examination of sterol crystals from pure ghee showed a shape of parallelogram similar to the one shown by pure cholesterol, while those from vegetable oil (groundnut oil) showed hexagonal form similar to the one shown by pure phytosterols. On the other hand, sterol crystals isolated from ghee samples adulterated with groundnut oil (15%) showed a characteristic crystal structure with re-entry angle (Swallow's tail), as shown by the mixture of pure cholesterol and pure phytosterol. Therefore, adulteration of ghee samples with 15 percent groundnut oil could be confirmed with this simple cost effective method. The presence of natural sterols in milk fat and the detection of adulteration with animal fat are the limiting factors of this method. Therefore, it can be inferred that microscopic examination of sterols structure can be used as a simple and cost effective tool for detecting added vegetable oils in ghee.

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SHORT COMMUNICATION

Study on consumer awareness of dairy analogues in Gujarat State

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Abstract: Milk is considered almost a complete food that provides macro and micronutrients in balanced proportions. To capture the market of milk and milk products, their imitation product manufacturers use the word 'milk'. A detailed survey on the consumer awareness of dairy analogues of 852 respondents from 8 districts of Gujarat was done. This study analyzed the consumer awareness towards dairy analogues, to determine whether price, taste, texture and push selling have a direct effect on market demand of dairy products and whether consumers pay attention to the labels of the products. It was concluded that the consumers are less aware of the dairy analogues (milk, ice cream, butter and cheese). They pay attention to the price primarily followed by taste, appearance and brand. The consumers do not pay much attention to the parameters such as nutritional benefits, labels, ingredients, and best before or use by date.

Keywords: Consumer awareness, Dairy analogue, Gujarat.

Milk has been an unquestioned staple food of the Indian diet for centuries. Milk is a nutrient-dense food of highly bioavailable nutrients at a very low cost; hence it is considered almost a complete food. It is the first food that is being received by any mammal after birth. The Codex Alimentarius Commission, the apex body for the collection of internationally adopted food standards and related texts, defines 'milk' as "The normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or further processing." In today's cut-throat competitive world, some imitation products are being tried to misguide and confuse consumers by systematic wrong marketing while using dairy terms like the word "milk." Many consumers are being diverted to plant-based products as non-dairy milk alternatives. In India, FSSAI (2017) notification has mentioned the use of dairy terms. Milk has been well defined as lacteal secretion; the term milk cannot be legally used for plant-based beverages or drinks. Under such circumstances, consumers must get real information backed by science and regulations. Balanced nutrition is an integral part of a healthy way of life. There is a global trend that the consumption of plant-based food products substitutes for the consumption of dairy products. There are abundant dairy analogues or imitation products available in the market today such as margarine or vegetable fat spreads, frozen desserts, filled cream, filled cheese and burfi are imitation products of butter, ice cream, dairy cream, natural cheese and sweetened khoa, respectively. Similarly, milk protein is substituted with vegetable proteins, especially soy proteins.

Food Safety and Standards Authority of India (FSSAI), an apex body for all matters related to food safety and standards in India defines milk as "The normal mammary secretion derived from complete milking of healthy milch animal, without either addition to that or extraction therefrom, unless otherwise provided in these regulations and it shall be free from colostrum." Analogues are the products manufactured using cheap substitutes either partially or wholly to the product's actual ingredient, e.g., milk fat, a unique and costliest ingredient of milk is replaced with more affordable alternatives such as vegetable fat/oils. There are plenty of imitation products available in the market and are wrongly marketed to target the consumers for more profit by manufacturers. The market of analogues in India is as high as 50,000 crores if we consider organized and unorganized sectors, said Vijay Sardana (Jitendra, 2018). Analogue/imitation products are physically and functionally similar products to the original product i.e. milk. The prime objective of the manufacturers of analogue/imitation products is to reduce the production cost, thereby fetching more profit by advertising the analogues as

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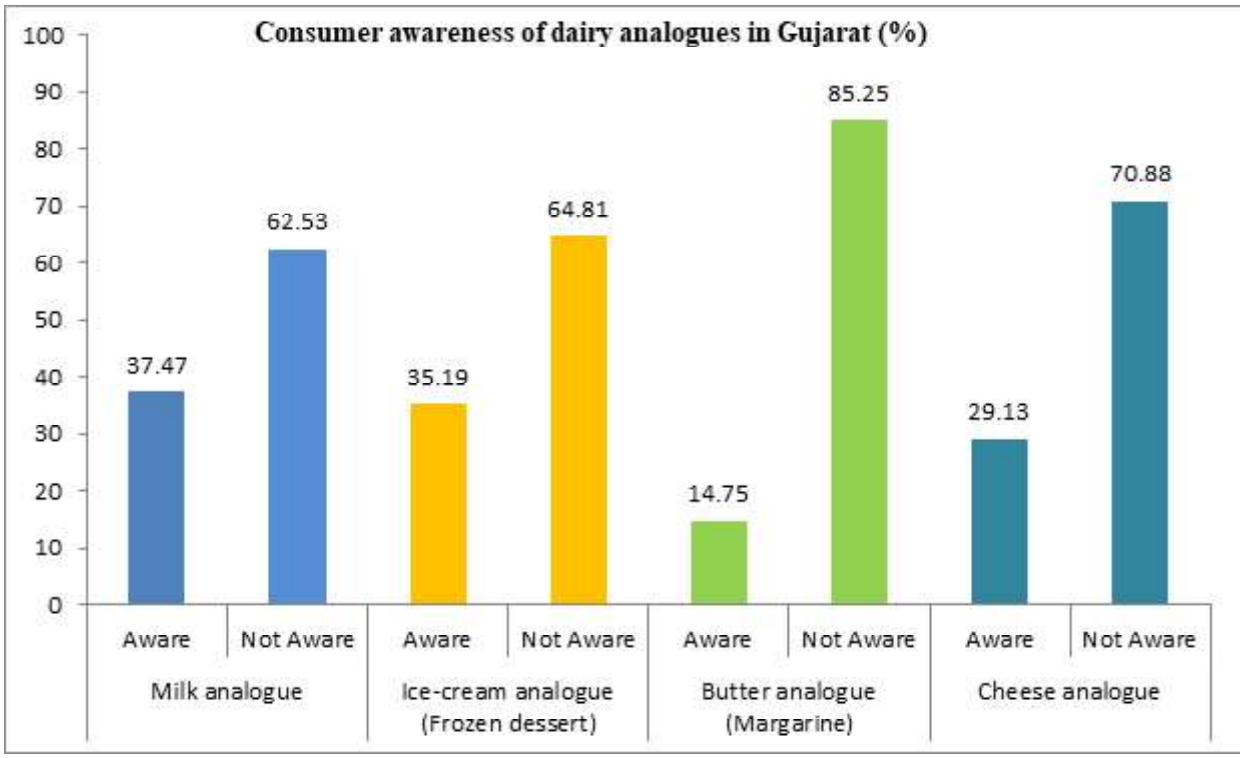

Fig 1: Consumer awareness of dairy analogues in Gujarat

healthier as and more functional than the original product. Dairy analogues are products that resemble milk-based products. Dairy alternatives, as opposed to animal-based products, are generally made out of plant-based ingredients. They are high in protein, nutritious vitamins, minerals, etc. They include products such as milk, butter, yoghurt, cheese and others. Milk includes products such as soy milk, coconut milk, almond milk, rice milk, oat milk, etc. Butter includes peanut butter, almond butter, etc. Yoghurt includes coconut milk yoghurt, cashew milk yoghurt, almond milk yoghurt, etc. Cheese includes treeline cheese, cashew cheese, etc.

There is a lack of systematic data on consumers' knowledge about the milk and milk products they purchase what they know about milk and its plant-based alternatives and their level of understanding about reading the labels, content, nutrients, etc. To arrive at the research investigation objectives, the team collected techno-social information from approximately 852 families from the Gujarat state. For a better representation of the state and different groups of consumers, survey sampling was done in both backward and forward districts from different regions of Gujarat - Banaskantha, Mehsana, Rajkot, Porbandar, Anand, Panchmahal, Surat, and Tapi. The survey was conducted in 8 districts of Gujarat from five regions viz., north, south, east, west and central Gujarat. From each district, the capital city, 2 towns and 4 developed villages were selected to survey the family level. Districts, towns and villages were selected in such a way that it gives a holistic picture of the society. Figure 1 shows consumer awareness of milk analogues. It is clear from the figure that the awareness of milk analogues is very less. Four different questions were asked to the households to check their awareness of milk analogues and it was observed that the overall awareness of milk analogues was only 37.47% while 62.53% of consumers are not aware of the milk analogues. It is clear from the figure that the awareness of Ice-cream analogues is also very less. Two questions were asked to the households to check their awareness of ice-cream analogues and it was observed that the overall awareness of ice-cream analogues was only 35.19% while 64.81% of consumers are not aware of ice-cream analogues such as frozen dessert. The figure shows the awareness of butter analogues is also very less. It was observed that the awareness of butter analogues was only 14.75% while 85.25% of consumers are not aware of the Butter analogues such as margarine. It is clear from the figure that the awareness of cheese analogues is also very less. It was observed that the overall awareness of cheese analogues was only 29.13% while 70.88% of consumers are not aware of the cheese analogues.

Conclusions

This research study investigated the perception of dairy analogues by real and potential consumers and determined the prospects of their understanding. It is clear from the observations that the consumers of Gujarat state are less aware of the dairy analogues despite their education level. There is a need to increase the awareness of dairy analogues amongst consumers.

Consumers pay attention to the price primarily followed by taste, appearance and brand which may affect the market demand for pure dairy products. It is evident from the result that the consumers are well educated but they are not paying much attention to the parameters such as nutritional benefits, labels, ingredients, and best before or use by date. Therefore, awareness campaigns at the pan India level are needed to increase the awareness amongst consumers. This project serves as a sample survey in Gujarat state. The data has been been shared with the policy makers of FSSAI. This paper can be a blueprint to launch the national-level survey across other states. There is a great need to study the consumer's perception. Further there is a need for the implementation of strict regulations by national regulatory bodies to reduce the impact of wrong branding and marketing of imitation products which captures the market of pure dairy products by using the brand equity of term milk.

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SHORT COMMUNICATION

Development of Ricotta cheese spread by using basket centrifuge

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Abstract: Maximum whey drainage is an important criterion in development of Ricotta cheese spread (RCS) as it ensures maximum removal of lactose from the cheese curd. Mechanization at small scale for preparation of RCS from Mozzarella cheese whey has been investigated by intervention in the whey drainage process using basket centrifuge. In the present study conventional method of whey drainage was replaced with basket centrifuge where whey drainage was carried out at different rpms (i.e., 1000, 1500, 2000 and 2500) for different time periods (i.e., 1, 2, 3, 4 and 5 min) and then moisture content in the curd was determined. The results indicated that with increase in centrifugal speed (from 1000 to 2500 rpm) and time period (from 1 to 5 min) there was a significant reduction in moisture content of the curd. The percentage reduction in the moisture content of cheese curd after centrifugation at 1000, 1500, 2000 and 2500 rpm for 5 min was observed to be 1.68%, 5.78%, 7.28% and 8.72% and corresponding yields of the curds were 7.2%, 6.33%, 5.86% and 5.13%, respectively. Curds obtained from basket centrifuge (at 2500 rpm for 5 min) and conventional methods were used to prepare the RCS and both the products were equally acceptable based on sensory evaluation. Use of basket centrifuge showed substantial attainment in reduction of time for the process of drainage of whey which is otherwise a time and capital intensive process.

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Keywords: Ricotta Cheese Spread; Basket Centrifuge; Whey Drainage; Moisture Content

Cheese is emerging as a fastest growing segment in dairy in India. The cheese market in India has been reported to reach a value of INR 56.7 billion in 2021 and expected to reach INR 211.5 billion by 2027 at CAGR of 24.3% during 2022-2027 (IMARC, 2022). Varieties of ripened and fresh cheeses are now being manufactured and sold in India viz. Cheddar, Mozzarella, Swiss, Gouda, etc. Whey, which is the major by-product of the cheeses, is known to be a nutritionally rich, containing large amount of organic matter such as whey proteins, lactose, minerals, etc. The gross composition of cheese whey on the basis of dry matter has been observed as 45-50 per cent total milk solids, 70-80 per cent lactose, 9-20 per cent proteins (mainly whey proteins), 8-20 per cent minerals (Kinsella and Morr, 1984; Hortan, 1995). Conversion of whey into nutritionally rich value added products can provide good opportunity to cheese entrepreneurs and consumers.

Whey cheeses are one such category which can be explored from the perspective of Indian consumers and cheese entrepreneurs and can be promoted both from commercial and nutritional point of view. Ricotta Cheese is a soft Italian unripened whey cheese known for its yellowish-white color and has a compact mass and granular texture (Ortiz Araque et al. 2018; Rubel et al. 2019). Ricotta cheese can be prepared with whey, milk, or milk/whey mixtures. The process of Ricotta cheese making involves co-precipitation of whey proteins and casein using suitable coagulant at suitable time-temperature combination and pH (Shelke et al. 2022; Lucey 2022). It is consumed fresh due to its susceptibility to microbial spoilage even under refrigeration condition, probably due to its high pH, high moisture content, low salt content, and water activity very close to unity (Martins et al. 2010). Bhatti et al. (2021) developed the probiotic Ricotta cheese in which limitations of Ricotta cheese developed from whey were discussed with special emphasis on prolonged time taken during drainage of whey from cheese curd and whey syneresis during storage of Ricotta cheese. To overcome the issue of whey syneresis in Ricotta cheese, hydrocolloids were added into the cheese followed by thorough blending and the cheese was converted into a spread like product known as Ricotta cheese spread (RCS). The overall process of making RCS takes

approximately 12-18 hours in which a major share of time was consumed during the whey drainage carried out under refrigerated conditions which makes it a time and capital intensive process as well as product becomes more susceptible to microbial growth. Therefore, in the present study, attempts have been made to use basket centrifuge as a whey drainage mechanism for cheese curd preparation for making RCS.

Mozzarella cheese whey (fresh and sweet) was collected during Mozzarella cheese preparation from the Experimental Dairy Plant (EDP) of the College of Dairy Science and Technology, Ludhiana. Standardized mixed milk (4.5% fat and 8.5% SNF) was also procured from the EDP of the college. Good quality common salt and citric acid were obtained from the local market for the preparation of RCS. Guar gum used as hydrocolloid was procured from HiMedia Laboratories, Mumbai, India. Total solids, fat, protein content and % titratable acidity of Mozzarella cheese whey and mixed milk samples were analyzed by method described in IS (1981). pH of the samples was determined by using digital calibrated hand pH meter (pH5, Cole Parmer, Mumbai, India).

Conventional method of Ricotta cheese was prepared as per the method described by Bhatti (2021). Sweet Mozzarella cheese whey was heated to 50°C to stop the growth of starter culture followed by addition of standardized mixed milk (4.5% fat and 8.5% SNF) with whey/milk mixtures of 80:20. The whey/milk mixture was heated to 90°C and when the temperature was attained, citric acid (5%, w/v) was added into it to acidify the mixture to pH 5.4. The flocculated protein rises to the surface and the curd along with Ricotta cheese whey (scotta) was allowed to rest for 20 minutes to complete the coagulation process. Later, curd was recovered and whey (also called scotta) was drained. In conventional method, curd obtained was hung overnight (14-15 h) for drainage using a clean muslin cloth under refrigerated condition (4°C).

Mechanized method for the manufacturing of Ricotta cheese was almost same as conventional except the whey drainage. Here whey drainage using muslin cloth was replaced with basket centrifuge with perforated cylindrical bowl (Deepali United Manufacturing Pvt. Ltd., Mumbai, India; capacity 200-500 g) carried out at different rpms (i.e., 1000, 1500, 2000 and 2500) for different time periods (i.e., 1, 2, 3, 4 and 5 min). Prior to use, the basket centrifuge and polypropylene bag which was fitted in the perforated cylindrical basket were cleaned with hot water at 80°C and dried. Moisture content of Ricotta cheese curd was analyzed as per the method described for cheese in IS (1981).

Ricotta cheese samples prepared by conventional and mechanized method were analyzed for its moisture content. The final moisture content of Ricotta cheese was adjusted to 75% for making RCS by using the formula given below.

$$X = (M_2\text{-}M_1/100\text{-}M_2) \times G$$

Where,

M_1= % Moisture present in Ricotta cheese curd

G = Weight of cheese in gram

M_2= % moisture required in RCS (75%)

X = Amount of water in gram to be added in Ricotta cheese curd

For the preparation of RCS, calculated amount of previously boiled water along with mixture of guar gum (0.4%) and common salt (1.5%) was added to the Ricotta cheese and further homogenized by using hand blender to produce smoother consistency. RCS was hot packed in pre-sterilized polypropylene cups (50 ml capacity) and stored at 4°C until further analysis.

A selected sensory panel assessed the coded RCS samples at random, according to the methodology described in Indian standards (IS, 1971). Sensory evaluation of samples was carried out with a 6-member panel who were scientists and students of College of Dairy Science and Technology, Ludhiana. The panelists had a good knowledge on the sensory evaluation of dairy products and participated previously in such evaluations. Samples were first prepared and then kept in a refrigerator at 4°C

Table 1 Effect of centrifugal speed and time on moisture content of Ricotta cheese curd prepared by basket centrifuge

Centrifugal speed (rpm)	Time (min)	Moisture content (%) in Ricotta cheese curd
1000	1	73.16±0.09^{a}
	2	73.12±0.06^{a}
	3	72.08±0.09^{c}
	4	71.83±0.11^{d}
	5	71.47±0.04^{e}
1500	1	72.83±0.07^{b}
	2	70.55±0.08^{f}
	3	68.94±0.05^{h}
	4	67.73±0.04^{j}
	5	67.49±0.05^{k}
2000	1	71.48±0.05^{e}
	2	68.42±0.06^{i}
	3	67.19±0.06^{l}
	4	65.72±0.02^{m}
	5	64.87±0.06^{o}
2500	1	70.05±0.06^{g}
	2	65.32±0.07^{n}
	3	62.62±0.08^{p}
	4	60.93±0.05^{q}
	5	59.74±0.09^{r}

Data are presented as Mean ± SD, n =3. Means with different lowercase superscripts are significantly different ($P < 0.05$) from each other.

Table 2 Effect of centrifugal speed on per cent moisture reduction and yield (%) of Ricotta cheese curd obtained by using basket centrifuge

Centrifugal speed (at different rpm for 5 min)	Moisture reduction (%) in cheese curd	Yield (%)
1000	1.68±0.03[d]	7.20±0.02[a]
1500	5.78±0.01[c]	6.33±0.02[b]
2000	7.28±0.04[b]	5.86±0.02[c]
2500	8.72±0.04[a]	5.13±0.07[d]

till sensory evaluation by panel. Each RCS sample was presented in a polypropylene cup filled with 20 g sample and labeled with a 3-digit code. Sensory evaluation of the samples was carried out in the sensory evaluation room under appropriate fluorescent lighting. Each panelist was asked to taste the samples and rate the sensory parameters on a 9-point hedonic scale and the acceptance test was carried out for the attributes of flavour, mouthfeel, colour and appearance, spreadability and overall acceptability. For spreadability parameter, panel members were provided with a spatula and bread and they were asked to spread the samples on the bread to evaluate the parameter.

Mean values and standard deviations (SD) of triplicate determinations were calculated using Microsoft excel (Microsoft office, 2010). All statistical analyses were performed using SPSS 16. One way analysis of variance (ANOVA) was used to determine differences among treatment means at 95% confidence interval.

Mozzarella cheese whey and mixed milk were used as raw materials at the ratio of 80:20 for preparation of Ricotta cheese curd followed by RCS. Mozzarella cheese whey contained 7.01% total solids, 0.78% fat and 0.9% protein whereas mixed milk contained 13.57% total solids, 4.5% fat and 3.52% protein. Higher titratable acidity (0.15% lactic acid) and lower pH (6.32) values were observed in Mozzarella cheese whey compared to full fat milk (0.13% lactic acid and pH 6.66).

Conventional method of whey drainage was replaced with basket centrifuge where whey drainage was carried out at different rpms (i.e., 1000, 1500, 2000 and 2500) for different time periods (i.e., 1, 2, 3, 4 and 5 min). The moisture content of Ricotta cheese was observed to be 70.94% when prepared by conventional method of whey drainage. On the other hand, with the use of basket centrifuge at different rpms, the moisture contents observed at 1000, 1500, 2000 and 2500 rpm at different time periods (from 1 to 5 minutes) were in the range of 73.16-71.47%, 72.83-67.49%, 71.48-64.87% and 70.05-59.74%, respectively (Table 1). The results indicated that with increase in centrifugal speed (from 1000 to 2500 rpm) and time period (from 1 to 5 min) there was a significant reduction in moisture content of the curd.

The percentage reduction in moisture content in Ricotta cheese curds after centrifugation for 5 min at different rpms using basket centrifuge is shown in the Table 2. The results clearly showed that with increase in centrifugation speed and time period, percentage reduction in moisture content of Ricotta cheese curd increased significantly ($p<0.05$). The Ricotta cheese curd obtained after centrifugation at 2500 rpm for 5 min showed maximum moisture reduction of 8.72%. On the other hand, the lowest moisture reduction of 1.68% was observed in the cheese curd after centrifugation at 1000 rpm for 5 min. Time taken for the process of removal of whey for the batch of 200-500 g using basket centrifuge was only up to 5 min. However, in conventional method, for the same process of whey removal it took up to 14-15 h for the same batch.

The yield of the Ricotta cheese obtained after centrifugation for 5 min at different rpms using basket centrifuge is shown in the Table 2. The per cent decrease in the yield of Ricotta cheese was observed with increase in the centrifugal speed from 1000 to 2500 rpm. The maximum yield (7.2%) in Ricotta cheese was observed at centrifugal speed of 1000 rpm whereas lowest yield (5.1%) at 2500 rpm. The difference in the yield percentages might be due to difference in the moisture contents of the curds obtained. Yield of ricotta cheese curds obtained are in agreement with the yield of ricotta cheese curd obtained with hanging method at same moisture content.

The study showed that basket centrifuge is an effective way of drainage of whey as we can achieve maximum reduction in moisture in the cheese curd with substantial reduction in time from hours to minutes. It is imperative to mention here that for preparation of RCS from Ricotta cheese maximum drainage of whey is an essential step as it ensures maximum removal of lactose from the cheese curd. Thus the product will be less susceptible to microbial spoilage and more it is suitable for lactose intolerant patients.

Sensory scores of RCS prepared by conventional method for flavour, mouthfeel, colour and appearance, spreadability and overall acceptability were 8.10±0.68, 8.15±0.74, 8.16±0.53, 7.48±0.70 and 8.13±0.57, respectively, whereas sensory scores of RCS prepared by mechanized method were 8.27±0.88, 8.30±0.57, 8.23±0.50, 7.51±0.48 and 8.23±0.47, respectively. The spread prepared with the Ricotta cheese curd obtained from basket centrifuge had no significant difference in the scores of all the sensory parameters and was identical to the spread prepared with Ricotta cheese curds obtained from conventional method.

The study concluded that the mechanization of the process can be adopted without affecting the sensory properties of the product.

Conclusions

Cheese industry is an emerging enterprise in India where varieties of cheeses are venturing into the market. RCS prepared from Ricotta cheese is one such novel product which has potential to be introduced in the upcoming market. New product development takes a lot of consideration when introduced into the market in terms of flavor, acceptability, cost, shelf life and ease of mechanization at large scale. One such step of mechanization at small scale at initial stages of study for RCS has been investigated by intervention in whey drainage process using basket centrifuge. Study showed that considerable amount of whey drainage from Ricotta cheese curd was possible at centrifugation of 2500 rpm for 5 minutes which showed some promising results. This study was carried out with a small batch of 10 liters of whey-milk mixture. An elaborative study may be taken up to standardize the process of Ricotta cheese and RCS in terms of microbiological, compositional, varying batch size, textural, cost and viability of this technology which might help in reducing the cost of the product and even help in designing the continuous production of Ricotta cheese and spreads.

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