Effect of loose house dairy cattle barn modification on udder health and production performance of Jersey crossbred cows in tropical lower Gangetic plains

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SUPPLEMENTARY FILE

MATERIALS AND METHODS-

Figure S1. Location of the experiment conducted

The present study was carried out at ICAR-National Dairy Research Institute (ICAR-NDRI), Eastern Regional Station, cattle yard located in Kalyani, West Bengal. The altitude of the city is 9.75 meter above mean sea level, latitude and longitude position being 22°56′30″N and 88°32′04″E, respectively. The weather of Kalyani is hot and humid; the maximum ambient temperature in summer goes up to 39°C and minimum temperature in winter comes down to about 8°C. The average annual rainfall is 1000-2000 mm, most of which is received from early June to September.

Details of housing modifications

The animals were kept under loose housing system divided into two different groups. Three sides of the paddock were surrounded by half brick wall and rest one side was fenced by manger and standing space. There was the drainage system in between covered and open space having adequate slope for better drainage. The standing space was moderate height and inclined with low slope. Water trough was provided in one corner of the paddock. This system of housing facilitated free movement and sufficient exercise to the animals. The housing despite the fact

that, exposes animals to climatic effects. The modified shed (experimental) was having sand bed floor (4-6 inches depth) and thatch ceiling (4 inches thick) under the asbestos roof, whereas existing shed (control) had concrete floor and asbestos sheet as roofing material. The measurements of floor space and roof thermal insulation area of modified house are given in the Table 1 and lay out diagram in Figure-1.

Table S1. Details on floor space and roof insulation provided to animals

Space (m^2)	**Control**	**Experimental**
Covered Area	L*B=8.5*6.75=57.38 m^2	L * B = 8.5*6.75= 57.38 m^2
Open Area	L*B = 11*8.5= 93.50 m^2	L*B = 11*8.5= 93.50 m^2
Total Area	L*B=17.75*8.5= 150.88 m^2	L*B = 17.75*8.5= 150.88 m^2
Total Sand Bed	-	L*B = 10.70*5.35= 57.25 m^2 **(37.94 % of total Area)**
Sand Bed under Covered area	-	L * B = 8.5*2.3= 19.55 m^2 **(34.07 % of Covered Area)**
Sand Bed under Open Area	-	L * B = 8.3*5.35= 44.41 m^2 **(47.50% of Open Area)**
Total Roof Area	L*B = 8.5*6.75= 57.38 m^2	L*B = 8.5*6.75= 57.38 m^2
Insulated Roof Area	-	L * B = 8.5*3.8= 29.75 m^2 **(51.85 % of Total Roof Area)**

Figure S2. Photograph of existing shed (control) of the farm

Concrete floor

Asbestos roof

Figure S3. Photograph of modified shed (treatment) with sand bed and paddy straw ceiling

Sand floor

Thatched ceiling under asbestos roof

Surface temperature measurement

Both floor and inside roof surface temperatures of shade materials were measured by infrared digital thermometer (-32°C ~ 320°C) of Metrix+^{TM} , MT 2A. Surface temperatures were taken

from 10 inches distance from the object. Surface temperatures were recorded 4 times in a day at 7:00-8:00 am, 10:00-11:00 am, 2:00-3:00 pm and 5:00-6:00 pm for 2 consecutive days, once in a week.

Milking practices, collection of milk sample from farm to perform milk tests and recording of total milk yield

The milking of experimental animals was done by machine milking method as routine practice being followed in the institute farm. Machine milking was done twice a day during morning from 6.00 to 8. 30 AM and evening from 2.30 to 4.30 PM. The milk was weighed and recorded in kg for individual animal. Before milking the animals were groomed and washed. Udders of cows were thoroughly washed with clean water just before the milking. Towels soaked with antiseptic solution were used for wiping of the udder and teats just before attaching the teat cups. Once in a week, morning milk samples were collected from all the experimental lactating cows. For this purpose, animal no. was written on the sample collecting bottles. Approximately 70 to 80 ml milk was collected aseptically in the clean and sterilized sampling bottles. Before taking the samples, the teats were thoroughly disinfected and allowed to dry and 1st 2-3 strips of fore milk were discarded. Modified California Mastitis Test (MCMT) was performed in the milking byre itself and milk smear for somatic cell count (SCC) were performed within one hour of collection. The samples were collected separately in sterilized sampling bottles and brought to the laboratory immediately for further analysis of milk fat and solid non-fat (SNF). Total milk yield during morning and evening hours were recorded at site of milking place and also cross checked register once in week from different groups of animal. Milk yield recording was done on morning and evening separately and added to obtain total milk yield of the day for each 20 animals. .

Modified California Mastitis Test (MCMT)

The MCMT test which is also known as cow side field test, was conducted in the milking byre itself during milking of cows as per procedure described by Devi (1989). The MCMT test is based on the principle of increasing in number of leukocytes and alkalinity of the mastitis milk. These changes are due to inflammatory exudation and increased contents of basic salts. Since the original Schalm reagent (Tri-ethanolamine sulphate and bromocresol purple) was not available, a

3% Sodium lauryl sulphate solution was prepared in the laboratory and used with same accuracy. This solution was prepared according to the method of Devi (1989).

Preparation of 3% sodium lauryl sulphate:

The test reagent prepared by adding 3 gm of sodium lauryl sulphate powder to 100 ml of distilled water. The suspension was mixed thoroughly by keeping it on heater and maintaining temperature of 50°C so as to make a clear solution. The pH of the solution was adjusted to 8.0 by using HCL or NaOH as per the need.

Test procedure:

A plastic paddle with four chambers or shallow cups was used to perform the test. Before taking milk samples in paddles, first 1-2 strips of milk was removed and then about 3 ml of milk was directly striped into the cups. Then approximately equal quantity of the test reagent was added in the cup. The mixture of the milk and reagent was shaken gently in a rotating manner of the paddle in the horizontal plane. Immediately after mixing, the reaction was observed. The reaction was graded by intensity of gel formation as described in (Table 2).

Table S2. Grading of SCM based on gel formation

MCMT-grade	**Description**	**Point**
N (Negative)	No change	'0'
T (Trace)	Slime formed which disappeared with continuous movement of paddle.	1
+ (Weak)	Distinct slime, but no gel formation.	2
+ + (Distinct positive)	Viscous with gel formation, adhered to the margin.	3
+ + + (Strong positive)	The gel formation with convex projection, the gel did not dislodge after swirling movement of the paddle.	4

Somatic Cell Count (SCC)

The Somatic Cell Count (SCC) in the milk was performed to assess the degree of intra-mammary infection in the respective quarters. The presence of SCC (leukocytes and epithelial cells) in the milk is directly indicates the level of inflammatory reaction of the infected quarter. The milk samples from the respective quarters as well as pooled were collected in sterile polyethylene screw capped wide mouth vials maintaining all aseptic precautions. The number of the cows and the quarter(s) from which the sample(s) collected was labeled accordingly on vials. The milk samples collected in vials were brought to the laboratory immediately for further examination. The smears of milk for SCC were prepared as early as possible (within one hour of its collection) to minimize disintegration of somatic cells.

Procedure:

The procedure for SCC in milk was adapted according to the method described by Schalm *et al.* (1971). Before making the smear, the test milk samples were thoroughly mixed by gentle shaking the vials and then 10µl (0.01 ml) of milk measured with micropipette, was taken on the pre drawn one square cm marked area over a grease free clean glass slide which was uniformly smeared with a standard sterilized bacteriological platinum loop and then the smears were kept for air dry. The slides were also labeled for respective animal and quarter.

Staining of milk smear:

The dried milk smears were then stained with the modified Newman's Lampert stain. The prepared slides were kept in the staining solution for 1 to 2 minutes and after 1-2 minutes the stain was drained off and the slides were kept for air dry. The smears were gently washed with tap water and then again dried. The dried stained milk smears were examined under the oil immersion lens of the microscope.

Counting of cells:

For counting of somatic cells, 30 different fields of milk smear were observed under oil immersion lens (100x) and the counting were repeated thrice per smear to assess average number of somatic cells in 30 fields and average number of the cells per field. The average number of cells per field was then multiplied by the microscopic factor of the microscope i.e. 240807 to obtain the number of cells per ml of the milk.

Determination Microscopic factor:

Microscopic factor for the present investigation was determined by the method illustrated by Shukla (1980). The diameter of the view field under 10x eyepiece and oil immersion lens was determined by using stage micrometer. The lowest division of micrometer scale was 0.01mm. Accordingly, the diameter was measured and obtained as 0.23mm. The area of the microscopic field was determined by the formula πr^2. The calculations were made as under:

Microscopic factor (MF) = Area of smear (in mm^2) / Area of the microscopic field

Area of the microscopic field:

Diameter = 0.23mm

Radius = 0.115mm

Area (πr^2) = $3.14 \times (0.115)^2$

= 0.0415265

Area of smear = 1 cm^2 ($100mm^2$)

So, MF = $100/3.14 \times (0.115)^2$

= 2408.07

Milk sample taken on the slide = 0.01ml

SCC/ml of milk = average number of cells per field× MF × 100

= 240807 × average number of cells per field

The same calibrated microscope was used throughout the course of study.

Estimation of milk Fat (%)

Estimation of fat percentage in milk was performed by adapting the Gerber's butyrometer method developed by Dr. N. Gerbers in 1892. The fat content is read directly via a special

calibrated butyrometer. Fat in milk exists in the form of an emulsion which is established by phospholipids and proteins. The principle of the Gerber method is based on the fact that the fat globules are de-emulsified by the addition of concentrated sulphuric acid (H_2SO_4) and fat get free from protein part. The free fat, with a lower density than the surrounding medium, may be separated rapidly by centrifugal force. Addition of Amyl alcohol speed up the process of separation of fat from the protein and gives a clearer dividing line on the butyrometer scale between the fat layer and the other material. Milk samples (temperature about 20 °C) were mixed thoroughly, taking care to minimize incorporation of air. Samples were allowed to stand for a few minutes to discharge any air bubbles. Now 10 ml of 90% by mass sulphuric acid was pipetted into the butyrometer, similarly, required volume of milk (10.75 ml) was also pipetted into the butyrometer gently. After adding milk, 1 ml of amyl alcohol was added into the mixture. The neck of the butyrometer was cleaned with tissue or dry cloth and the butyrometer was Stoppered tightly using a clean and dry stopper. Then it was shaked and inverted several times until all the milk reacts with acid. Now the butyrometer was centrifuged for 5 minutes at 1100 rpm. Fat percentage was read by bringing the graduation mark to eye level. If necessary, the fat column was adjusted by regulating the position of the stopper.

Preparation of 90% H_2SO_4:

Preparation of 90% H_2SO_4 is required to estimate the fat (%) in milk sample. Conc. H_2SO_4 of 90 ml was taken in a 100 ml measuring cylinder and added 10 ml distilled water in it slowly in H_2SO_4 with the stirring the solution, make up the final volume 100 ml. Prepared solution stand for cooling at room temperature, make the final volume by adding some drop of distilled water.

Estimation of milk SNF (%)

Milk SNF percentage was estimated by lactometer method using the ISI formula. Lactometer reading was taken through the ISI calibrated lactometer at 27^0C. The corrected lactometer reading (CLR) was calculated by adding or subtracting the correction factor to lactometer reading depending upon the milk temperature.

ISI Formula

$$S.N.F. = \frac{CLR}{4} + 0.25 \times (Fat\ \%) + 0.44$$

Estimation of Total Solid (%)

Total Solid = Fat + S.N.F

Table S3. Analysis of variance showing the effect of treatment and seasons on daily milk yield in kg (No of animals: N=20, No of observation: n=2993)

Source of variation	Df	Mean sum square	F value	Level of Significance
Groups / treatment	1	151.70	11.90	0.001
Season	1	528.34	41.46	0.000
Groups × Season	1	100.97	7.92	0.005
Error	2989	12.74	-	-

Table S4. Analysis of variance showing the effect of treatment and seasons on Fat (%), SNF (%) and Total Solid (%) in milk (No of animals: N=20, No of observation: n=395)

Source of variation	Df	Mean sum square	F value	Level of Significance
Fat (%)				
Groups / treatment	1	7.99	19.85	0.000
Season	1	0.001	0.002	0.960

Groups × Season	1	0.21	0.53	0.468
Error	391	0.40	-	-
Solids Not Fat (SNF) (%)				
Groups / treatment	1	0.20	0.91	0.339
Season	1	16.29	74.20	0.000
Groups × Season	1	1.30	5.92	0.015
Error	391	0.22	-	-
Total solid (%)				
Groups / treatment	1	5.66	6.64	0.010
Season	1	16.55	19.43	0.000
Groups × Season	1	0.46	0.54	0.462
Error	391	0.85	-	-

Table S5. Analysis of variance showing the effect of treatment and seasons on MCMT (grade) in Jersey crossbred cows (No of animals: N=20, No of observation: n=94)

Source of variation	**Df**	**Mean sum square**	**F value**	**Level of Significance**
Groups / treatment	1	4.73	5.99	0.016
Season	1	0.61	0.77	0.382
Groups × Season	1	0.05	0.06	0.803
Error	90	0.79	-	-

Table S6. Analysis of variance showing the effect of treatment and seasons on Number of Somatic cell count /field, Somatic cell count (in Lakh) / ml of milk and Log_{10} SCC / ml of milk in Jersey crossbred cows (No of animals: N=20, No of observation: n=94)

Source of variation	**Df**	**Mean sum square**	**F value**	**Level of Significance**
Number of Somatic cell count /field				
Groups / treatment	1	10.76	3.42	0.068
Season	1	2.19	0.70	0.407
Groups × Season	1	0.27	0.09	0.769
Error	90	3.15	-	-
Somatic cell count (in Lakh) / ml of milk				
Groups / treatment	1	62.37	3.42	0.068
Season	1	12.69	0.70	0.407
Groups × Season	1	1.59	0.09	0.769
Error	90	18.25	-	-
Log_{10} SCC / ml of milk				
Groups / treatment	1	0.27	1.47	0.229
Season	1	0.003	0.02	0.897
Groups × Season	1	0.10	0.52	0.474
Error	90	0.19	-	-