ISSGPU

Vol. 25(1) April, 2019

The Indian Journal of Small Ruminants

NAAS Rating 5.25

Print ISSN 0971-9857
Online ISSN 0973-9718

An Official Publication of

Indian Society for Sheep and Goat Production and Utilization

THE INDIAN JOURNAL OF SMALL RUMINANTS

Vol. 25(1) **April, 2019**

Contents

Review and Overview

1. **Potential use of waste wool in agriculture: an overview**
S.C. Sharma, A. Sahoo and Roop Chand ---------- 1

Research Papers

2. **Genetic diversity and bottleneck analysis of sheep based on microsatellite markers**
R. Selvam and P. Kathiravan ---------- 13
3. **Selection indices for improvement in body weights and fleece yield of Chokla sheep**
Prakash, S.B.S.Yadav, Urmila Pannu, A. Chopra, H.K. Narula, A.K. Patel ---------- 19
4. **Milk yield potential of prolific Avishaan and GMM sheep and their association with overall ewe productivity**
Ved Prakash, R.C. Sharma, L.L.L. Prince and Arun Kumar ---------- 25
5. **Population structure and effect of inbreeding on lamb growth in close flock of Bharat Merino sheep**
P.K. Mallick, R. Venkataramanan, S.M.K. Thirumaran, S. Rajapandi, G. Nagarajan, G. Murali and A.S. Rajendiran ---------- 31
6. **Factors affecting the relative growth rate of Pantja kids under field conditions**
B.S. Khadda, Brijesh Singh, D.V. Singh, S.K. Singh and C.B. Singh ---------- 37
7. ***In vitro* fertilization of sheep oocytes with Patanwadi ram semen**
Basanti Jyotsana, Krishnappa B, S. Bahire, A.S. Meena, D. Kumar, S.M.K. Naqvi and S. Kumar ---------- 41
8. **Influence of fecundity gene on oestrus synchronization and gonadal steroid levels in Booroola carrier and non-carrier ewes**
S.V. Bahire, Kalyan De, D. Kumar, Meena Kataria and S. Kumar ---------- 45
9. **Blood biochemical metabolites of post-parturient goats under different management systems in Himalayan region**
S. Shafiq, R.A. Patoo, H.M. Khan, A.A. Shah, A. Fayaz and S.S. Nissa ---------- 50
10. **Effect of supplementary feeding of sheep ration pellets and sprouted black-eyed beans (*Vigna unguiculata*) on milk intake and weight gain in suckling Barbados Blackbelly lambs**
S.A. Ali, U. Krishnamoorthy, G. Kathiravan and A. Paul ---------- 54
11. **Risk factor analysis for neonatal lamb mortality at an organized farm of arid Rajasthan**
C.P. Swarnkar, H.K. Narula and A. Chopra ---------- 59
12. **Prevalence of *Cryptosporidium* species in goats of Namakkal, Tamil Nadu**
S. Shanmathi, G. Ponnudurai, N. Rani and K. Sukumar ---------- 70
13. **Clinicopathological study of ruminal acidosis in small ruminants**
Rahul Kumar, Neeraj K. Gangwar, Barkha Sharma and Arvind Tripathi ---------- 75
14. **Micrometrical studies of lung in prenatal Gaddi sheep**
Brij Vanita, Rajesh Rajput and Virender Pathak ---------- 80
15. **Comparative study on meat composition and fatty acid profile of non-descript and their crosses with Boer goats**
S. Das, G.P. Mohanty, S.K. Mishra, S.P. Sahoo, S.K. Dash, A.R. Sahu and C.R. Pradhan ---------- 85
16. **Use of natural plant proteases in tenderization of mutton**
Y.P. Gadekar, A.K. Shinde, G.G. Sonawane, V.K. Saxena and S.V. Bahire ---------- 89
17. **Effect of blending fine and medium coarse wools on blanket quality**
N. Shanmugam, Seiko Jose, Ajay Kumar, Sekhar Das and D.B. Shakyawar ---------- 95
18. **Impact of Mega sheep seed project on reproduction and mortality in Mecheri sheep flocks**
S. Senthilmuthukkumaran, K. Sivakumar, V. Ramesh and A.K. Thiruvenkadan ---------- 99
19. **Economic analysis of commercial goat farming in Tamil Nadu**
M. Gunaseelan, B.P. Singh, Mahesh Chander and D. Bardhan ---------- 103

Indian Journal of Small Ruminants 2019, 25(1): 89-94

Indian Journal of Small Ruminants

USE OF NATURAL PLANT PROTEASES IN TENDERIZATION OF MUTTON

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Manuscript received on 04.08.2018, accepted on 31.08.2018

DOI: 10.5958/0973-9718.2019.00024.2

ABSTRACT

In the present study wild *kachri (Cucumis trigonus Roxb:* wild melon) powder and papain were used to improve tenderness of meat of old animals. Various tenderizing treatments [per cent water/tenderizing agent to meat weight (w/w)] for mutton chunks were control (10% distilled water), T1 (2.5% kachri powder), T2 (2.25% kachri powder + 0.25% papain) and T3 (0.25% papain) for 48 h at refrigeration storage. The pH, moisture and ash contents of mutton chunks were comparable. Significantly higher ($P<0.05$) water holding capacity (%) was observed in T1 (35.19±1.40) than T2 (25.77±0.61) and T3 (30.79±2.54) samples. Shear force values (N/cm^2) were reduced significantly ($P<0.05$) in T1, T2 and T3 groups (28.62±1.75, 28.41±1.73, 18.99±1.33) than the control (69.74±5.36). The myofibrillar fragmentation index was significantly higher ($P<0.01$) in the T1, T2 and T3 groups (62.36±2.24, 54.93±3.04 and 48.84±3.65) than the control (24.95±2.35). The protein (mg/g) and collagen solubility (% total collagen) were significantly higher in tenderized samples (T1: 134.84±2.08 and 87.41±1.00), (T2: 134.72±1.81 and 85.74±4.73) and (T3: 133.66±3.81 and 82.94±2.78). The lightness values significantly ($P<0.05$) decreased due to the tenderization treatment. Significantly ($P<0.05$) higher sensory scores (juiciness, tenderness, connective tissue residue left and overall palatability) were observed in the treatment groups. Histological examination of muscle fibres and SDS-PAGE of muscle protein revealed that 2.5% kachri treated samples showed more degradation of muscle fibres. The findings of the study indicated that 2.5% kachri powder could be used for tenderization of tough meat from cull sheep.

Key words: *Cucumis trigonus*, Mutton, Papain, Tenderization

Tenderness of meat is an important quality attribute and number of pre-and post-slaughter factors affects it. Mutton produced from cull sheep is tough and not desired and accepted by the consumers. In order to utilize tough meat, tenderization is the better option. Nowadays enzymatic tenderization of meat by plant proteases is the most common method. Proteases from plant sources like, papain, bromelain, fincin and other protease sources are used. The fruit (*kachri*) of *Cucumis trigonus Roxb*; wild melon) plant has protease activity and is used for tenderization of meat. Very limited literature is available on the tenderization of cull sheep meat using kachri powder, papain, and their combination Therefore a study was undertaken to evaluate the efficacy of kachri fruit and papain individually and in combination as a meat tenderizer.

MATERIALS AND METHODS

The study was undertaken during June-December, 2013. *Bicep femoris* muscle from six female Malpura sheep (6-7 year-old) was procured from Livestock Products Technology Section, ICAR-Central Sheep and Wool Research Institute, Avikanagar. Mutton was kept in chilled condition for 24 h. Mutton chunks (~3-4 cm thick) were made from it and used in the study. Mature fruits kachri (*Cucumis trigonus Roxb*) were collected from the local market.

After thoroughly washing fruits were cut and seeds were removed. Further, they were oven dried at 40±2°C for 72 h and fine powder was made in a domestic mixer. Papain was procured from Sisco Research Laboratories Pvt. Ltd. India.

Uniform-sized (3-4 cm) mutton chunks were marinated [percent water/tenderizing agent to meat weight (w/w)] either with control (10% distilled water), or T1 (2.5% kachri powder), T2 (2.25% kachri powder +0.25% papain) and T3 (0.25% papain). The treated chunks were kept in a glass bowl at 4±1°C for 48 h and afterwards the mutton chunks were washed and drained. Part of chunks were cooked at 80°C in an oven for 20 min and used to evaluate sensory attributes. The raw samples were used for physico-chemical and histological studies.

For estimation of pH, 10 g of sample was mixed with 50 ml distilled water and pH was measured using a digital pH meter (Oakton, pH Tester 30). Moisture and crude protein contents were estimated as per AOAC (1995). The water-holding capacity (WHC) was estimated by the filter paper press method (Grau and Hamm, 1953, 1957). Shear force values (kg/cm^2) were measured for cores of $1cm^3$ sizes from cooked meat samples. Protein solubility was measured as per the method of Joo et al. (1999). Sarcoplasmic protein was determined by the biuret method. Total protein (sarcoplasmic + myofibrillar) was derived from 2 g sample using 40 ml ice-cold 1.1 M potassium iodide in 0.1 M phosphate buffer (pH 7.2). The difference between total and sarcoplasmic protein indicated myofibrillar protein concentration. The method of Davis et al. (1980) was followed to determine the myofibrillar fragmentation index (MFI) with slight modification. Hydroxyproline of the meat sample was measured by the procedure of Nueman and Logan (1950). The hydroxyproline content was arrived at by using a standard graph. Collagen content was determined by using 7.14 multiplication factor. The method described by Mahendrakar et al. (1989) was used for collagen solubility and calculated as:

$$\% \text{ Collagen solubility} = 7.14 \times \% \text{ hydroxyproline solubilized}$$

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was done as per Laemmli (1970) and See and Jackowski (1993). A Jaypak CR331C Colorimeter (Minolta Camera Co., Japan) having light source was D65 and a 10° standard observer was used for assessing colour attributes of the chunks. Readings were obtained from different locations of sample, eluding large bits of connective tissue or fat particles. Semi-trained panelists (8 no.) using 8–point descriptive scale evaluated appearance, flavour, juiciness, tenderness and overall palatability (Keeton, 1983). Data were subjected to analysis of variance and means were compared by Duncan's multiple range test to assess the effects between samples (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The results of physico-chemical attributes of raw mutton chunks are presented in Table 1. The pH, moisture and ash contents of the mutton chunks were comparable. Similar results have been reported by Gadekar et al. (2011) in case of meat pH of the cull sheep. The moisture and ash contents of all the groups were comparable. The WHC of mutton chunks was significantly ($P<0.05$) lower in the chunks treated with a combination of kachri and papain powder. In the present study it was higher than those reported by Naveena et al. (2004). Significantly ($P<0.05$) higher WHC in T1 might be due to higher yield of collagen on hydrolysis of connective tissues (Xiong 1997). The shear force values were significantly ($P<0.05$) reduced by the treatments as compared to the control and might be due to proteolytic effect causing damage to cross-links in the muscle fibre (Zochowska-Kujawska et al., 2013). Similar results of decrease in shear force values by incorporation of ginger extract / powder in sheep meat (Mendiratta et al., 2000) and spent hen meat (Naveena, and Mendiratta 2001) have been reported.

The myofibrillar fragmentation index was significantly ($P<0.01$) higher in the treatment groups and might be ascribed to the proteolytic action of the tenderizing treatment which caused more fragmentation in chunks compared to control chunks (Zhao et al., 2012). Tenderness and fragmentation index were positively correlated. Lower myofibrillar fragmentation index indicates tough meat (Kandeepan et al., 2009). The sarcoplasmic, myofibrillar protein and collagen solubility were significantly (P<0.05) higher in tenderized samples and might be attributed to the collective effect of changes in pH, reduction in the number of net positive charges, salt, and disintegration of myofibrils and collagen caused due to tenderization effect, selective binding of ions, as salt alters the isoelectric point of meat proteins (Puolanne and Halonen, 2010). Mendiratta et al. (2010) also observed increased protein solubility due to ginger and papain in spent hen and cull sheep meat, respectively. Further, Naveena et al. (2004) reported ginger extract increased the collagen solubility, sarcoplasmic and myofibrillar protein solubility in treated buffalo meat samples.

Table. 1. Physico-chemical attributes (Mean±S.E.) of raw mutton chunks

Attribute	Control	T1	T2	T3
pH	5.43±0.02	5.47±0.07	5.40±0.01	5.35±0.01
Moisture (%)	75.43±0.28	74.86±0.49	75.11±0.2	75.13±0.24
Ash (%)	1.42±0.27	1.62±0.17	1.43±0.17	1.23±0.08
Water holding capacity (%) *	28.25±3.09[b]	35.19±1.40[a]	25.77±0.61[b]	30.79±2.54[ab]
Shear force value (N/cm^2) *	69.74±5.36[a]	28.62±1.75[b]	28.41±1.73[b]	18.99±1.33[c]
Myofirbillar fragmentation index **	24.95±2.35[c]	62.36±2.24[a]	54.93±3.04[ab]	48.84±3.65[b]
Sarcoplasmic protein solubility (mg/g)*	28.87±0.66[c]	33.55±0.72[a]	33.85±0.05[a]	31.23±0.56[b]
Myofibrillar protein solubility (mg/g)*	83.87±1.09[b]	101.29±2.61[a]	100.88±1.77[a]	102.43±3.78[a]
Total protein solubility (mg/g)*	112.74±1.03[b]	134.84±2.08[a]	134.72±1.81[a]	133.66±3.81[a]
Collagen content (mg/g of tissue)	6.05±0.42	6.21±0.02	5.65±0.42	5.21±0.11
Collagen solubility (% total collagen)*	39.95±1.52[b]	87.41±1.00[a]	85.74±4.73[a]	82.94±2.78[a]
L (lightness)*	61.91±0.8[a]	60.35±0.27[b]	59.34±0.13[bc]	58.7±0.16[c]
a (Redness)	3.38±0.2	3.03±0.13	3.13±0.04	3.47±0.09
B (Yellowness)	12.82±0.52	12.64±0.15	13.25±0.09	13.15±0.16
Hue	15.44±1.8	13.71±0.54	13.51±0.24	15.09±0.42
Chroma	13.28±0.44	13±0.15	13.62±0.08	13.6±0.16

Control- 10% distilled water; T1- 2.5% kachri powder; T2- 2.25% kachri powder +0.25% papain; T3- 0.25% Papain; Means bearing different superscripts within row differ significantly (- P<0.05, ** P<0.01); n=6 for moisture and ash, 3 for colour attributes, 12 for* water holding capacity *and 22 for shear force value*

The colour attributes of the mutton chunks revealed that the lightness values were significantly (P<0.05) reduced while redness, yellowness, hue and chroma were unaffected due to tenderization treatment. Similar results were reported by Narsaiah et al. (2011) for goat meat on the use of pomegranate rind powder as a tenderizer. The results of physico-chemical and sensory qualities of cooked mutton chunks are presented in Table 2. The,control, kachri and papain-treated cooked mutton chunks showed significantly (P<0.05) higher pH values. Moisture and ash contents of the meat chunks were comparable. The results of sensory evaluation of mutton chunks indicated that scores for juiciness, tenderness, connective tissue residue left and overall palatability were significantly (P<0.05) higher for the treatment groups.

Table 2. Physico-chemical and sensory attributes (Mean±S.E.) of cooked mutton chunks

Attribute	Control	T1	T2	T3
Physico-chemical (n=3)				
pH	5.69±0.02[a]	5.70±0.02[a]	5.71±0.01[a]	5.61±0.00[b]
Moisture	67.56±1.63	69.69±0.60	67.71±0.31	66.62±0.16
Ash	1.61±0.18	1.61±0.16	1.77±0.13	1.57±0.35
Sensory attributes (n=15)				
Appearance	6.13±0.20	6.06±0.19	6.19±0.21	6.25±0.25
Flavour	5.69±0.24	6.13±0.27	5.75±0.25	5.94±0.30
Juiciness	4.63±0.41[b]	6.06±0.29[a]	5.88±0.39[ab]	5.50±0.56[ab]
Tenderness	3.94±0.40[b]	6.22±0.27[a]	5.69±0.37[a]	5.94±0.48[a]
Connective tissue residue	4.06±0.44[b]	6.19±0.20[a]	5.69±0.35[a]	5.72±0.45[a]
Overall palatability	4.69±0.44[b]	6.16±0.16[a]	6.03±0.32[a]	5.81±0.45[a]

Control - 10% distilled water; T1 - 2.5% kachri powder; T2 - 2.25% kachri powder +0.25% papain, T3 - 0.25% Papain; Means bearing different superscripts within row differ significantly (P<0.05)

The improved juiciness in the tenderized samples might be attributed to the disintegration of muscle fibres and thereby holding more water between the spaces. Improvement in tenderness score was evident by reduction in force required for shearing muscle fibres. The scores for connective tissue residue left are due to the disintegration of connective tissue due to the activity of the plant proteases. Improvement in juiciness and tenderness scores due to plant protease treatment in the present study is similar to the findings of Naveena et al. (2004). The tenderness of the cooked meats is improved by hydrolyzed collagen derived from the connective tissues (Badr, 2008). The scores for overall palatability were significantly (P<0.05) higher in treated mutton chunks. A similar effect on sensory attributes was reported on tenderization with plant proteases by Zochowska-Kujawska et al. (2013).

SDS–PAGE analysis showed a decrease in the protein bands in treated meat chunks suggesting better proteolysis of muscle proteins (Plate 1). Enzyme-treated samples showed disruption of high molecular weight proteins into 30 kDa or lower molecular weight proteins (Huff-Lonergan et al., 1996), which caused an augmented concentration of low molecular weight protein bands. Augmented proteolysis in enzyme treated meat chunks might be linked with significantly (P<0.05) more protein solubility. Jorgova et al. (1989) opined that when muscle proteins are treated with bacterial proteolytic enzyme, there is a decrease in the number of higher molecular weight segments due to disintegration of myosin resulting in improved tenderness.

Histological examination of muscle fibre (Plates 2 to 5) revealed that 2.5% kachri treated samples showed more degradation of muscle fibres. The microstructure of buffalo muscle treated with ginger extract indicated that muscle fibres were broken into different bundles shown by increased the gaps between the bundles (Naveena et al., 2004). Similarly, elastase from the Bacillus strain when used in beef showed apparent distortion and an interruption of structure (Chen et al., 2006).

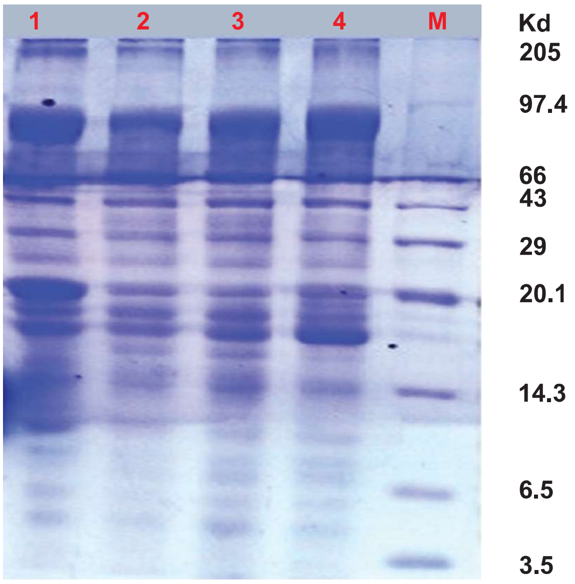

Plate 1. Electrophoretic pattern of muscle proteins treated with proteases (Lane 1- control; lane 2-2.5% Kachri powder treated sample; lane 3-2.25% Kachri powder+0.25% papain treated sample; lane 4-0.25% papain-treated sample; lane M - molecular weight marker)

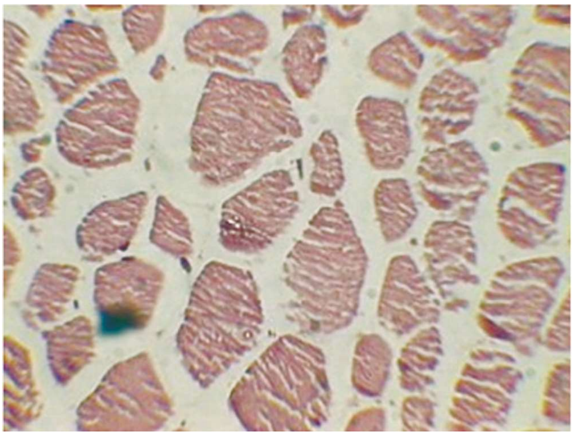

Plate 2. Histological changes in control meat sample (H&E stain 40X)

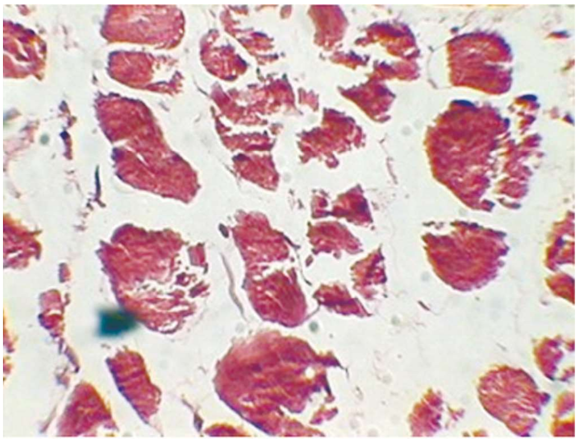

Plate. 3. Histological changes in 2.5% Kachri powder treated meat sample (H&E stain 40X)

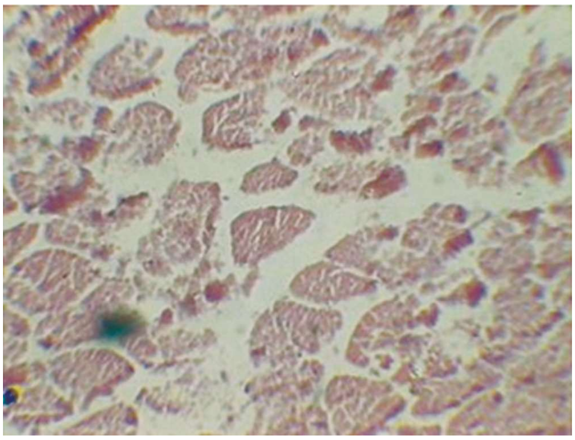

Plate 4. Histological changes in 2.25% Kachri powder + 0.25% papain treated meat sample (H&E stain 40X)

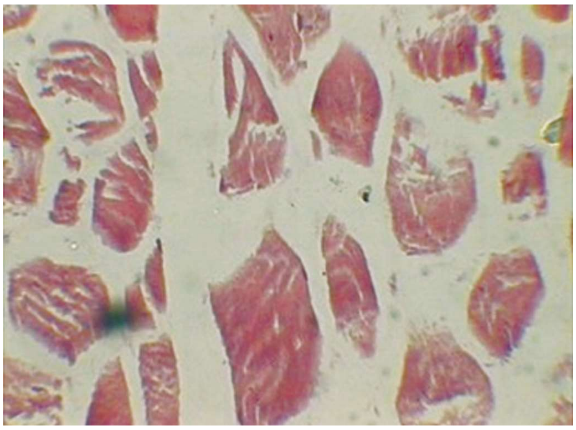

Plate 5. Histological changes in 0.25% papain-treated meat sample (H&E stain 40X)

The findings of the present study indicated that 2.5% (w/w) kachri powder could be efficiently utilized for tenderization of tough meat from cull sheep without any harmful effect on other meat quality attributes. The findings of the study are also useful since the low-value tough meat from cull sheep could be efficiently utilized. Consequently, a technology for effective use of easily and modestly available kachri fruit could be explored commercially for tenderization of tough mutton.

ACKNOWLEDGMENTS

We are grateful to the Director, ICAR-Central Sheep and Wool Research Institute, Avikanagar for providing facilities for the research work. The help from Dr. R.S. Bhatt, Principal Scientist and Mr. S.A.Q. Naqvi, Senior Technical Officer, ICAR-Central Sheep and Wool Research Institute, Avikanagar during the study is gratefully acknowledged.

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Indian Journal of Small Ruminants 2019, 25(1): 95-98

Indian Journal of Small Ruminants

EFFECT OF BLENDING FINE AND MEDIUM COARSE WOOLS ON BLANKET QUALITY

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Manuscript received on 24.04.2018, accepted on 21.06.2018

DOI: 10.5958/0973-9718.2019.00003.5

ABSTRACT

An attempt was made to use medium coarse wool separately as well as in blends with fine wool for the preparation of blankets with an objective to give value addition to medium coarse wool by way of blending. One fine wool sheep - Bharat Merino (BM) and two medium coarse wool sheep – Chokla (CH) and Avikalin (AV) were selected for the study. Three types of yarns were prepared from Bharat Merino, Chokla and Avikalin after scouring and carding of raw wool. Yarn count was kept at 4.0 Nm for all the yarns. Six types of blankets were prepared from three types of yarn with ends/inch of 22 and picks per inch of 19 with 525 gsm. In the tensile characteristics, maximum breaking load of 318 N was observed for CH blanket followed by BM / CH blanket (292 N). Thermal insulation value of blankets ranged from 2.68 to 3.88 Tog. In smoothness ranking of blankets, the ranks in the decreasing order of smoothness were: BM, BM+CH, CH, BM+AV, AV+CH and AV. From the point of view of smoothness and tensile characteristics it can be concluded that one can blend 50% BM with 50% CH for optimum blanket quality.

Key words: Blanket, Friction coefficient, Smoothness, Tensile, Wool

Wool is the preferred raw material for the preparation of eco-friendly blankets. Woollen blankets made from fine wool give smooth feel but are expensive. Woollen blankets made from coarse wool having a diameter of 45.8 μ using charka and pit loom in Coimbatore district of Tamil Nadu and blankets were found to have harsh feel and was suggested to add fine wool to improve the quality (Raja et al., 2008). Literature review has revealed that there exists research gap in understanding the effect of blending medium coarse wool with fine wool in making of blankets. Blending of coarse wool with fine wool will help to reduce the cost of the product and also add value to the coarse and medium coarse wool. Hence, an attempt was made to use medium coarse wool separately as well as in blends with fine wool for the preparation of blankets.

MATERIALS AND METHODS

One fine wool sheep - Bharat Merino (BM) and two medium coarse wool sheep – Chokla (CH) and Avikalin (AV) were selected for the study. BM and AV wools were obtained from Southern Regional, Research Centre, Mannavanur, Tamil Nadu while CH was obtained from Arid Research Centre, Bikaner, Rajasthan of ICAR-Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan. The study was conducted at ICAR-CSWRI, during June to December 2017. Raw wool was tested for wool fibre diameter and percentage of medullated fibres using the standard test method IS 744: 2000. Staple length was tested as per IS 6653: 1972. Bundle strength was measured using stelometer at 0 mm gauge length and crimps per cm were measured using steel scale and visual counting.