

celebrating  
50 years  
CSWRI - AVIKANAGAR

# Research Contributions 1962 - 2012



केन्द्रीय भेड़ एवं ऊन अनुसंधान संस्थान

अविकानगर - 304 501 राजस्थान

Central Sheep and Wool Research Institute  
Avikanagar - 304 501 Rajasthan



## HEADS OF THE INSTITUTE

S. No	Name	From	To
1	Dr M.V. Krishna Rao	04.01.1962	28.09.1964
2	Dr O.N. Singh	29.09.1964	05.12.1968
3	Dr M.L. Kohli	06.12.1968	19.02.1969
4	Dr A. Roy	20.02.1969	23.01.1970
5	Dr B.D. Patil	24.01.1970	15.12.1970
6	Dr R.M. Acharya	16.12.1970	20.06.1978
7	Dr C.L. Arora	21.06.1978	25.03.1979
8	Dr R.M. Acharya	26.03.1979	11.06.1981
9	Dr C.L. Arora	12.06.1981	11.05.1982
10	Dr R. Nagarcenkar	12.05.1982	04.06.1984
11	Dr Manohar Singh	05.06.1984	22.07.1984
12	Dr C.L. Arora	23.07.1984	04.06.1985
13	Dr B.C. Patnayak	05.06.1985	24.11.1986
14	Dr A.L. Chaudhary	25.11.1986	26.01.1990
15	Dr B.C. Patnayak	27.01.1990	30.11.1995
16	Dr S.C. Sharma	01.12.1995	22.03.1996
17	Dr R.N. Singh	23.03.1996	31.08.2000
18	Dr R.C. Jakhmola	01.09.2000	08.10.2000
19	Dr B.U. Khan	09.10.2000	30.06.2002
20	Dr V.K. Singh	01.07.2002	31.01.2007
21	Dr A.L. Arora	01.02.2007	19.02.2007
22	Dr S.A. Karim	20.02.2007	Continue



# Research Contributions 1962 - 2012



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अविकानगर-304 501 राजस्थान  
**Central Sheep and Wool Research Institute**  
**Avikanagar-304 501 Rajasthan**

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Published By

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## Preface

The Central Sheep and Wool Research Institute (CSWRI) is a premier Institute of Indian Council of Agricultural Research (ICAR) engaged in research, training and extension activities on sheep and rabbits production and value addition of their produce. It was established in 1962 at Malpura in Rajasthan, now campus is known as Avikanagar. The campus is spread over an area of 1510 ha in hot semiarid region of the country. It has three Regional Research Centers in different climatic zones of the country for developing region specific technologies. North Temperate Regional Station (NTRS) was established in 1963 in temperate region at Garsa, Kullu in Himachal Pradesh. The Southern Regional Research Centre (SRRC) was established in 1965 in sub-temperate region at Mannavanur in Tamil Nadu. Arid Research Campus (ARC) was established in 1974 at Bikaner in hot arid region of Rajasthan. The Institute and its sub-stations have been working for enhancing the productivity of sheep and rabbit by developing scientific practices and new technologies. The main motto of institute is to enhance productivity of sheep and rabbit for livelihood security, economic sustenance of farmers and wholesome and hygienic meat for consumers and wool for woollen industries in the country. The research achievement of institute was first compiled in 1987 on the occasion of Silver Jubilee year of the institute. Second publication was brought out on 35 years of research contribution of institute. The present publication on the occasion of Golden Jubilee Year of the institute is being brought out incorporating the progress made in research and technologies developed for benefit of stakeholders during the last 50 years.

The Institute has made considerable progress in genetic improvement of native sheep breeds, development of prolific sheep, improvement of feed resource and its utilization, development of feeding practices and nutrition approaches for enhancing mutton production, improving reproductive efficiency, disease resistance, disease diagnostics and surveillance, developing carpet, technical textiles and apparels woollen products, value added meat products and transfer of technologies. The research contributions of Central Sheep and Wool Research Institute, Avikanagar over the last five decades, more specifically for livestock farmers in harsh and difficult topographies and their adoption brought about visible improvement of farmer's income by increasing productivity of sheep and reduction of economic losses due to morbidity and mortality.

The present publication is prepared in view of newer challenges faced currently in sheep production and those expected in the near future under evident climate change and shrinking grazing resources, intermixing of native breeds, emergence and re-emergence of diseases, marketing trend of wool and meat, slump in wool price and sharp rise in mutton price.

It is expected that the analytical approach and forward looking concepts presented in the publication will prove useful for the researchers, policymakers, and stakeholders to address the future challenges for growth and development of the sheep sector and ensure food and income security with a human touch. On the occasion, I thank all the scientists for their contribution in bringing out publication on the occasion of Golden Jubilee Year of the institute. I express my gratitude to Dr S. Ayyappan, Secretary, DARE and Director General, ICAR, Ministry of Agriculture, New Delhi for his invaluable guidance in the working of the Institute. I am grateful to Prof K.M.L. Pathak, DDG (Animal Science) and Dr S.C. Gupta, ADG (AP&B), ICAR, New Delhi for their keen interest and generous support in the activities of the Institute. I appreciate the efforts of Dr A.K. Shinde, Dr C.P. Swarnkar and Dr L.L.L. Prince in bring out this document on the occasion of Golden Jubilee Year of the institute.



(S.A. Karim)



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## About the CSWRI

The Central Sheep and Wool Research Institute (CSWRI) is a premier Institute of Indian Council of Agricultural Research engaged in research, education and extension activities on sheep and rabbits. It was established in 1962 at Malpura in Rajasthan, now campus is known by the name of Avikanagar. The campus is spread over an area of 1510 ha in hot semiarid region of the country. It has three Regional Research Centres in different climatic zones of the country for developing region specific technologies. North Temperate Regional Station (NTRS) was established in 1963 in temperate region at Garsa, Kullu in Himachal Pradesh. The Southern Regional Research Centre (SRRC) was established in 1965 in sub-temperate region at Mannavanur in Tamil Nadu. Arid Research Campus (ARC) was established at Bikaner in hot arid region of Rajasthan during 1974. The Institute and its sub-stations have been working for enhancing the productivity of sheep and rabbit by applying scientific practices and developing new technologies. The mandate of Institute is to undertake research, training and transfer of technology for improving meat and wool production of sheep and rabbits and to develop processing technology of sheep and rabbit products.

### Mandate

Basic and applied research on sheep and rabbit production, health, utilization, training and transfer of technologies to the beneficiaries

### Objectives

- ❖ To undertake basic and applied research on all aspects of sheep and rabbit production,
- ❖ To develop, update and standardize meat, fibre and pelt technologies,
- ❖ To impart trainings on sheep and rabbit production and utilization,
- ❖ To transfer improved technologies on sheep and rabbit production to farmers, rural artisans and development workers and
- ❖ To provide referral and consultancy services on production and products technology of sheep and rabbits.

### Linkages

Institute has been regularly involved in research and development of technologies through linkage with following ICAR Institutions, SAUs, SVUs, Development Boards and other research organizations. Presently, the projects are running in the mode of AICRP, Network, NAIP, Sheep and Wool Improvement Scheme (CWDB), IICP (DBT, ICMR), NICRA, NFBSFARA etc.

ICAR Institutes	State Universities	Other Organizations
IVRI, Izatnagar	RAJUVAS, Bikaner	CWDB, Jodhpur
NDRI, Karnal	SKUAST-K, Jammu and Kashmir	DBT, New Delhi
CRIDA, Hyderabad	MPKV, Rahuri	ICMR, New Delhi
IGFRI, Jhansi	SVVU, Palamner	DST, Jaipur
CIRG, Makhdoom	SDAU, Dantiwada	
NIANP, Bangalore	TANUVAS, Chennai	
NRCE, Hisar	OUAT, Bhubaneshwar	
	BAU, Ranchi	
	KVAFSU, Bidar	



## Animal Genetics and Breeding

**A.L. Arora, L.L.L. Prince and A. Chopra**

Sheep Genetic Section was established in 1966 to conduct research on improvement of indigenous breeds of sheep for wool and meat production. The section was upgraded to Division of Animal Genetics and Breeding in 1975. During 1960's the sheep improvement programme through cross breeding was started by importing Rambouillet sheep from Texas (USA). Subsequently during 70's Soviet Merino sheep were imported from Stavropol (erstwhile U.S.S.R.) for cross breeding the indigenous medium wool producing sheep for fine wool production. Dorset and Suffolk sheep were imported for crossbreeding the indigenous sheep to evolve new mutton type breeds. Malpura and Jaisalmeri sheep were crossed with exotic Rambouillet. Several crossbreds of different grades were produced and evaluated for their growth performance, wool yield and quality traits. In 1971 projects for fine wool and mutton production were initiated in form of All India Coordinated Research Project. Later the two projects were merged into a single project on Sheep Breeding with fine wool component and mutton component. In Mutton component, the crossbreeding of local Malpura and Sonadi sheep with exotic Suffolk and Dorset was initiated in 1974 to evolve mutton type sheep to attain body weight of 30 Kg at 6 months of age. In the fine wool component, Chokla and Nali sheep were crossed with Rambouillet as well as Soviet Merino to produce good quality apparel wool bearing sheep. The biochemical polymorphic studies were also undertaken to study the inheritance of various blood constituents and to determine the possible association of these biochemical parameters with economic traits. Marwari, Sonadi and Malpura breeds were crossbred with Karakul to produce lambs for pelt production. Research was also carried out with indigenous goats to evolve chevon-producing goats so as to attain 25 kg body weight at 6 month of age. Studies on cytogenetics were initiated in 1982. In 1994 Awassi sheep was introduced in the mutton project with the aim to improve milk and body weight. Awassi was crossed with native Malpura sheep. In 1997, Garole, a prolific microsheap of West Bengal was introduced for increasing prolificacy of Malpura sheep. In 2008, Patanwadi inheritance was introduced into prolificacy project to improve the milk yield and mothering ability. In 2010, Kendrapada, another prolific sheep from Odisha was introduced in to the prolific sheep project. The important achievements related to crossbreeding works of the division are summarized below:

- 1963 Import of Romney Marsh, Southdown and Rambouillet sheep
- 1971 All India Coordinated Research Projects on sheep were started, Import of Soviet Merino sheep from Stavropol (erstwhile U.S.S.R.),  $\frac{3}{4}$  Rambouillet crosses of different indigenous breeds produced
- 1974 Import of Dorset and Suffolk sheep
- 1975 Import of Karakul from USSR
- 1976 Avikalin and Avivastra strains of sheep evolved
- 1983 Mutton Synthetic (Avimaans) strain of sheep evolved
- 1986 Bharat Merino strain evolved
- 1992 Conversion of AICRP in to Network Project on Sheep Improvement
- 1994 Introduction of Awassi sheep
- 1995 Awassi X Malpura half-breds sheep were produced
- 1997 Introduction of Garole sheep
- 1998 Garole X Malpura half-breds were produced

- 2005 ¾ Malpura ¼ Garole crosses (GMM and MGM) produced
- 2008 Introduction of Patanwadi inheritance in the Prolific sheep project
- 2009 Three breed crosses (GMM X Patanwadi and Patanwadi X GMM) produced  
Mega Sheep Seed Project Initiated
- 2010 Introduction of Kendrapada sheep in the Prolific sheep project

Crossbreeding with exotic germplasm was used as a tool for genetic improvement in mutton and wool production. A number of new breeds/strains for mutton, fine wool, superior carpet wool and pelt production have been evolved through interbreeding and selection of halfbreds. Crossbreeding results indicated that the superiority in body weight, greasy fleece weight and fleece quality of newly developed strains over the indigenous purebreds involved in crosses. Same level of performance was not obtained in the extensive system of management in farmers flock. Crossbred strains had relatively lower reproductive efficiency and survival. Performance of synthetic strains was satisfactory in favorable sub-temperate climatic conditions. Keeping in view of the results obtained from crossbreeding experiments, selective breeding of important indigenous sheep breeds was taken up. At present Malpura, Chokla sheep and Sirohi goat are maintained and selective breeding for improving the production potential is in progress. Program is also in progress to introgress *FecB* gene from Garole or Kendrapada sheep in to non prolific Malpura sheep and to evolve a prolific sheep strain.

### Carpet wool production

**Avikalin:** A crossbreeding experiment for improving wool production was initiated at CSWRI, Avikanagar in 1964-65 involving exotic fine wool breed (Rambouillet) and native extremely coarse wool breed (Malpura). Since the beginning of 1975 the half bred have been pooled and interbred and the new strain arising out of this base having 50% Rambouillet and 50% Malpura inheritance was named as *Avikalin* (*Avi*- Sanskrit word for sheep and *Kalin*- a Persian word for carpet). Avikalin was evolved in 1977 with the objectives to produce 2 kg annual greasy fleece yield (GFY) with ~ 30 µ fibre diameter and ~ 30% medullation. During the initial years and up to 1992 ram lambs/breeding rams were selected following the sequential selection based on weaning, 6 and 12 month weights, 1<sup>st</sup> six monthly GFY and six monthly GFY and wool quality traits (viz. staple length at 6 month age and diameter at yearling stage). The objectives of wool quality traits were achieved and since 1993 efforts were made to fully exploit the production potential of Avikalin sheep and to develop as promising dual type sheep. Towards this, selection was made based on an index incorporating 6-month body weight and 1<sup>st</sup> six monthly GFY. Under the project "Improvement and testing of Avikalin sheep", available Avimaans ewes were mated with Avikalin rams for upgrading and subsequent merging with Avikalin flock. Avikalin X Avimaans (CM) was further upgraded with Avikalin and 3CM having 87.5% of Avikalin was produced. Progeny from the crosses of 3CM and Avikalin were merged with Avikalin.



The quality of wool produced by Avikalin sheep is of carpet quality. Wool has been found useful even for preparation of serge and blending with other fibres for superior quality hosiery. Avikalin is more superior in economic returns through the sale of wool alone compared with Malpura. Least square means of first six monthly GFY and adult annual GFY was 1.034 kg and 1.560 kg during the period 2010-11. Avikalin produces wool with average fibre diameter and medullation of around 30µ and 30%, respectively with staple length of 4.5 cm. The least square means of body weight at birth, weaning, 6 and 12 month were 3.03, 15.07, 26.56 and 33.56 kg, respectively. The annual tuppung and lambing (available basis) was 93.67 and 84.39%, respectively. Overall survivability is maintained at above 90%. This indicates that the Avikalin sheep was well adapted to the local climatic conditions.

Performance of Avikalin sheep was also tested in arid climatic conditions in Bikaner. Body weights at 6 and 12 month were 20.83 and 26.00 kg, respectively. Lambing percentage was 72.9. GFY at spring and autumn clip was 814 g and 1202 g, respectively. It shows that Avikalin has adapted to arid conditions with performance similar to semi-arid conditions. To evaluate its performance in sub-temperate climate, a small flock of Avikalin was transferred to SRRC, Mannavanur (Tamil Nadu). Growth and GFY performance are much higher than the performance of Avikalin sheep in semi-arid climate. Body weights at 6 and 12 month were 25.23 and 37.5 kg, respectively. Adult annual GFY was 2.512 kg. Compared to Malpura, Avikalin had shown 12.5% improvement in 6-month weight and 90% in annual GFY. Higher average daily gain (ADG) in pre- and post-weaning phase indicates good mothering ability in Avikalin sheep. Avikalin can be used as an improver breed for cross breeding the coarse wool breeds of sheep to increase carpet wool production. Results clearly indicate that Avikalin sheep has surpassed the performance of Malpura sheep in all the traits of economic importance and it can be very well used as dual type sheep for carpet and mutton production.

**Chokla:** A fine carpet wool sheep breed of Rajasthan primarily reared for its superior quality carpet wool and its suitability for migration. The AICRP on sheep breeding was initiated in 1971. Since then Chokla was the one of the native breed used for cross breeding with exotic animals for developing different crossbred such as Avivastra and Bharat Merino. From 1991 AICRP on sheep breeding has been converted into Network Project on Sheep Improvement and the project "Evaluation and improvement of Chokla sheep for carpet wool" was started in April 1992 and still continuing aiming to improve wool yield. Overall least-squares mean during the start of the project (1992-95) for body weight at birth, weaning, 6 and 12 months of age were 2.77, 11.81, 16.51 and 21.35 kg, respectively. Corresponding growth performance during the period 2004-08 were 2.76, 13.90, 19.58 and 25.33 kg, respectively. Animals were kept under semi-intensive management system over the years. Over the period there is significant improvement in the body weight at different ages and this reflects the effect of selection in the flock. Six and 12 month body weights of 24.83 and 30.29 kg, respectively were achieved with change in concentrate supplementation regime during the period 2009-11. Overall least square means in 2009-11 for first six monthly GFY and Adult annual GFY were 1.438 and 2.386 kg, respectively. Being a Best Carpet wool breed, Chokla produces wool with average fibre diameter and medullation of around 30 $\mu$  and 30% with staple length of more than 6.0 cm suitable for all kind of carpet preparation. Since 1992, a total of 250 rams were sold to the farmers / Government of Rajasthan / NGOs for breed improvement programme.



### Fine wool production

**Bharat Merino:** Bharat Merino is a strain of fine wool sheep evolved by crossing the half-bred ewes of Chokla, Nali, Malpura and Jaisalmeri with Rambouillet and/or Russian Merino rams and stabilizing the population at 75% exotic fine wool inheritance. Native sheep viz. Chokla- a fine carpet wool breed, Malpura -a coarse and hairy wool breed and Jaisalmeri- a medium carpet wool sheep breed were mated with Rambouillet for production of half-breds. Half bred ewes were backcrossed with Rambouillet rams for production of  $\frac{3}{4}$  Rambouillet. A similar project involving crossbreeding of native Chokla and Nali ewes with Rambouillet and Russian Merino rams from USSR was in progress under AICRP since 1971 in the Institute. The  $\frac{3}{4}$  crosses of both the projects were merged in 1982. After merging these were inter bred and named as Bharat Merino. Performance of Bharat Merino sheep was satisfactory with regards to production and reproduction except that in semi-arid condition it has not been possible to obtain more than 5 cm staple length at six monthly shearing. Keeping this in view, in 1987 Bharat Merino sheep were transferred at SRRC, Mannavanur to evaluate its performance in sub-temperate climate. The performance of Bharat Merino was significantly improved with regards to production and reproduction and the problem of short staple length at six month was over come by shearing animals annually.

As given below, the performance of Bharat Merino sheep with respect to production, reproduction and adaptability were significantly higher in sub-temperate climate of Mannavanur than semi-arid climate of Avikanagar.

Parameters	Avikanagar	Mannavanur
<b>Wool traits</b>		
Greasy fleece yield (kg)	2.05±0.10	2.61±0.11
Staple length (cm)*	3.24±0.05	8.11±0.07
Fiber diameter (μ)	17.94±2.25	19.38±0.10
Medullation (%)	2.66±0.33	0.79±0.13
<b>Body weights (kg)</b>		
Birth	3.38 ± 0.01	4.10±0.01
3 months	15.97±0.07	18.40±0.09
6 months	23.36±0.09	25.33±0.13
12 months	30.60±0.11	32.30±0.35
<b>Reproductive performance (%)</b>		
Tupping	89.68	91.48
Lambing (available basis)	71.63	82.31
<b>Survivability (%)</b>		
0-3 months	93.18	97.17
3-6 months	96.29	98.40
6-12 months	92.00	98.72
Adult	94.10	97.91



\*At Avikanagar Staple length at six month and at Mannavanur annually

The strain has the potential as an import substitute for Rambouillet and Merino rams to be used as improver breed for improving the indigenous breeds for carpet wool and fine wool production. Improved rams of Bharat Merino sheep are in demand in states of Himachal Pradesh, Haryana, Tamil Nadu, Karnataka and Jammu and Kashmir.

**Avivastra:** Chokla ewes were crossed with Rambouillet rams to produce half-breds, 5/8<sup>th</sup> and 3/4<sup>th</sup>. Rambouillet x Chokla F1, F2 and 5/8<sup>th</sup> were grouped together and given name of Avivastra in 1977. The half-breds were interbred to stabilize exotic inheritance at 50% and further improvement in greasy fleece weight and quality was brought through selection. The half-breds and 5/8<sup>th</sup> crossbreds of Rambouillet and Merino with Chokla and Nali were interbred. It resulted in Chokla and Nali Synthetic strains in 1983. The performance of both the strains was similar. During the year 1989-90 the groups of Avivastra and fine wool synthetics (Chokla and Nali Synthetic) sheep were merged together into a single group 'Avivastra' after comparing groups for greasy fleece weight, staple length, medullation percentage and economic traits.

Production performances with respect to birth, weaning, 6 and 12 month weight were 3.04, 14.77, 19.80 and 24.43 kg, respectively. Adult annual GFY was more than 2.5 kg. Breed possessed all the parameters best fit with fine wool quality. Survivability in pre-weaning, post-weaning and adult sheep was 92.78, 97.85 and 95.28%, respectively. Wool quality parameters of Avivastra and Bharat Merino crosses are quite comparable except the medullation %, which was higher than the Bharat Merino. Avivastra sheep meet most of the requirements for apparel wool traits although medullation percent is slightly higher. Due to poor acceptability and reduced reproductive performance of Avivastra sheep in the field it was decided to upgrade Avivastra sheep by crossing with rams of Bharat Merino and

merging in with Bharat Merino. All the upgraded Avivastra sheep were merged with Bharat Merino in year 2000. After upgrading Avivastra sheep using Bharat Merino, upgraded rams can be used as an improver germplasm for improving coarse fibre breed for improving wool quality. The crossbred wool is suitable for processing on worsted system.

### Mutton production

**Avimaans:** The Avimaans strain of sheep has been evolved through crossbreeding of Malpura and Sonadi ewes with imported Dorset and Suffolk rams. AICRP on sheep breeding for mutton production was initiated in 1970-71 with the objective to evolve new mutton breed(s) suitable for semi-arid conditions of the country, which would have an average body weight of 30 kg at 6 month of age. Against this objective, an average weight of about 34 kg could be achieved in Mutton synthetic subsequently named as 'Avimaans'. Overall means for birth, 6 and 12 month body weight were 3.31, 20.50 and 28.45 kg, respectively. Most of the feedlot trials indicated higher body weight gain and efficiency of feed conversion in Avimaans compared to native breeds (Viz. Malpura and Sonadi). On an average, Malpura lambs took 122 and 138 days to achieve 20 and 25 kg body weight whereas mutton synthetic lambs took 108 and 122 days, respectively. Mutton synthetic lambs had better ADG (179 g) than Malpura (158 g). In Avimaans the tugging was low as compared to the natives and it varied from 55.98 to 86.9% in different years. Annual lambing on the basis of ewes available was 58.2%. The first 6 monthly and adult 6 monthly GFY were 0.64 and 0.57 kg, respectively. However due to higher fat percent in carcass, the objective was revised to attain 25 kg body weight at 4 to 4½ months of age. In view of relatively poor reproductive performance of Avimaans, its further multiplication was stopped and it was decided to improve Malpura sheep for mutton production.

**Awassi x Malpura (AM):** Considering the importance of heavy body weight and milk yield in economic mutton production, a pilot project on crossbreeding of Awassi rams with Malpura ewes was started in 1994, at CSWRI, Avikanagar. AM half-bred lambs exhibited improvement over Malpura lambs at birth, 3, 6 and 12-month weights. These crossbreds produced more 1<sup>st</sup> six monthly wool yield compared to Malpura lambs. Crossbred wool was found to have better wool quality compared to Malpura wool. AM ewes produced 29% more milk than that of Malpura ewes. Farmers were fascinated by fat tail of Awassi and they showed interest in crossbreeding of native Malpura ewes with Awassi rams. Least-square means for birth, 3, 6, 9 and 12 month body weight were 3.42, 14.71, 22.12, 26.01 and 29.43 kg, respectively. Results revealed that AM crossbreds exhibited 18.75, 27.05, 19.56, 19.59 and 14.92% improvement over their native counterparts of Malpura breed. The superiority trends of AM lambs were observed at weaning stage could not be sustained after weaning despite having production potential which indicated that the crossbreds require more inputs in terms of feed and fodder to maintain the superiority trend. Feed conversion efficiency was higher (19.8%) in AM than Malpura lambs (15.7%). The Awassi crosses had better growth performance (150 g ADG) than the Malpura (115 g ADG) lambs.



The overall least-squares means for 1<sup>st</sup> six monthly, adult six monthly and annual adult GFY were 0.794, 0.561 and 1.022 kg, respectively. AM crossbreds produced 37% more 1<sup>st</sup> six monthly GFY compared to Malpura lambs. The AM wool was found to have better wool quality with 36µ diameter and 45% medullation compared to 45µ diameter and 60% medullation in Malpura wool. Crossbred wool was also lustrous and could be used as carpet wool. Awassi x Malpura ewes produced 42% more milk than that of Malpura ewes. Average daily milk yield was 501 g. The annual tugging and lambing on tugged basis was 86.21 and 71.79%, respectively. AM crossbred were phased out in year 2002 due to non-availability of Awassi rams. Awassi is a milch breed in its native country and the purpose of crossing was to produce a milch sheep breed although milk from sheep is not of any commercial importance in the country at present. But there is a great scope of using sheep milk for producing specialty cheese and other dairy products having scope for more value addition.

**Malpura:** Malpura, an indigenous sheep, is well adapted to harsh climatic conditions of semi-arid region. It is an important mutton type breed with coarse wool texture and found in Jaipur, Tonk and Sawai Madhopur districts of Rajasthan. Ewes are having sufficient milk to sustain their neonates. Malpura being a native breed has been used in almost all the crossbreeding programs being run in the CSWRI to produce various crossbred for mutton, fine wool and carpet wool improvement like Avimaans, Avikalin, Bharat Merino, Awassi x Malpura crosses and Indian Karakul. Selective breeding in Malpura sheep is going on to genetically improve the breed for mutton production. Simultaneously, an elite flock of pure Malpura is also being built up. The rams of Malpura are supplied to the farmers for improving their sheep flock. There is always a high demand of superior rams in local sheep farmers to improve their own flock. Previously, the young rams were ranked and selected on the basis of an index incorporating six-month weight and 1st six monthly GFY. Selection index has been revised to give due advantage to the animals having better growth. At present Selection index is based on index incorporating six month body weight, pre and post weaning average daily gain. The performance Malpura sheep is as under:

Traits	1974-77	2010-2011
<b>Growth performance (kg)</b>		
Birth weight	2.52±0.03 (288)	3.08±0.02 (520)
Weaning weight	9.74±0.17 (234)	15.58±0.17 (369)
Six month weight	13.84±0.29 (116)	25.74±0.24 (237)
Twelve month weight	19.46±0.39 (64)	31.90±0.31 (181)
<b>Greasy fleece yield (kg)</b>		
First six monthly	0.506±0.04 (32)	0.619±0.013 (223)
Adult annual	0.771±0.02 (226)	0.802±0.019 (325)
<b>Wool quality</b>		
Average fibre diameter (μ)	39.41±0.41 (145)	54.62±1.99 (19)
Medullation (%)	82.70±0.21 (145)	90.82±2.30(19)
Staple length (cm)	5.76±0.06 (145)	7.48±0.34 (19)
<b>Reproduction (%)</b>		
Tupping	94.07 (135)	93.64 (393)
Lambing (Available basis)	70.87 (135)	85.75 (386)
<b>Survivability (%)</b>		
0-3 month	91.21	95.01(521)
3-12 month	92.14	97.41(425)
Adult	79.07	96.40(751)



Tremendous improvements (86%) in Malpura sheep have been observed at market weight (6 month) when compared to performance during 1974-77. This improvement is realized due to genetic selection made on six month body weight over the period and coupled with improvement in feeding and managerial interventions. Survivability always remained more than 95% in the flock. High genetic merit Malpura rams are being supplied to the interested farmers for genetic improvement of their animals. About 50 Malpura rams every year are being sold to the farmers for genetic improvement of their flock.



### Prolific sheep

Improved animal genetic resources with versatile characteristics are the need of the day to meet the growing human demand. India is endowed with wide diversity of sheep genetic resources, which forms the backbone of its rural livelihood security systems. Sheep rearing now faces a dilemma to produce more mutton and wool for the growing human population against the reality of shrinking grazing resources, creating a major constraint to the further growth of sheep population. In the present scenario the demand for meat in India has increased rapidly and the emphasis has shifted from wool towards mutton as the main produce from sheep rearing. There is acute shortage of meat for domestic needs besides huge demand in the international market. The gap between the demand and production of mutton could be bridged by augmenting the reproductive rate of low producing Indian sheep breeds. Improving prolificacy in sheep flock can increase the profitability of sheep production. Lamb production has become a most important source of income for sheep rearer. In order to improve the fecundity of sheep, incorporation of genetic material of prolific sheep is an ideal approach to evolve a large size breed capable of multiple births for economic and remunerative mutton production.

Most of the domestic sheep breeds of the world are reported to deliver only one or two lambs at each lambing. The increase in production associated with litter size is controlled by both genetic and environmental factors. Since the heritability of litter size is low in sheep, attempts were made to discover the gene(s) controlling ovulation rate and thus litter size (LS). Realizing the importance of *FecB* mutation, extensive work has been done all over the world including India improving prolificacy and its effects and economics in sheep production traits. CSWRI, Avikanagar, also took the opportunity to conduct the research on prolificacy introgression from Garole sheep into native Malpura sheep. A cross breeding scheme was initiated in 1997 to introgress the *FecB* gene from India's most valuable germplasm "Garole sheep" of a hot and humid environment into the non-prolific and large size mutton sheep breed "Malpura" best adapted of a semi-arid tropical environment to produce the Garole x Malpura (GM) crossbred carrying *FecB* gene. Encouraging results have been obtained in GM half-breds in terms of twinning rate and growth of quarter-breds (GMM; backcross of GM with Malpura). The viable benefits accrued by gene infusion are reflected by 45.7% increase in the prolificacy and 35.05% in weaning rate of GM compared to that of Malpura. A single mutated copy of *FecB* gene produced 0.70 and weaned 0.58 extra lambs in GM sheep. The improved *FecB* carriers (BB and B+) are proposed to be sold to the interested sheep farmers for making genetic improvement and increasing prolificacy in their flocks without compromising with the growth rate.

Having developed prolific GM half-bred sheep, there was scope to exploit the prolific GM sheep for relatively better growth by backcrossing with native Malpura. In the backcrossing programme the *FecB* gene carrier GM rams were used as sires and Malpura ewes as dam breed to produce the GM x Malpura (GM (M); 75% Malpura and 25% Garole) and reciprocal crosses were also attempted to produce M (GM). The 3/4<sup>th</sup> crosses so produced are able to produce twins and also have higher body weight compared to half-breds but problem of low milk yield from the dam persisted in the 3/4<sup>th</sup> crosses. In year 2009 Patanwadi sheep inheritance was introduced in the GM (M) to increase the milk yield of dams. In this process, 3 breed crosses are being developed which will result in higher prolificacy, higher birth weight along with more milk yield of dam to feed larger lamb crop per ewe.

**Garole x Malpura (GM):** There was a tremendous increase in multiple births and in other reproductive efficiency traits in GM ewes compared to Malpura ewes. The results provided the first evidence for increased litter size in GM, with the possibility of further exploitation using appropriate breeding and selection strategies. The average litter size at birth was 1.56 with highest litter size of 1.68 was rerecorded in year 2008 and 2010. There was significant improvement in multiple births in GM crosses compared to Malpura. The GM ewes had 49% (1.56 vs 1.05) advantages over Malpura for litter size at birth. Average twinning and triplet in last 6 years was 40.09% and 9.80%, respectively in GM. The growth performance of GM in terms of body weights recorded at birth, 3, 6, and 12 months age were 1.87, 10.13, 15.27 and 20.59 kg, respectively.

It was obvious that on crossing the small sized Garole rams with large sized Malpura ewes the half-bred progenies would weigh less than the dam parent and higher than the sire parent. Two reasons contributed to these differences, one; the fact that the cross contained genes from a significantly lighter breed, the second; many of the crossbred lambs were born twins or triplets which are mostly lighter than single-born lambs. The pre-weaning survivability ranged from 87.94 to 93.36. Overall survivability of the GM over the years was greater than 89.90%. These results further indicated that the progenies of GM crossbred were inheriting traits of adaptation from Malpura sheep and the prolific genes from Garole. In January 2009 Garole x Malpura (GM) female (ID 504) weighing 27.4 kg at lambing gave birth to triplets, (two males and one female lamb). Litter weight of 6.40 kg at birth, 36.40 kg at three month and 62.60 kg at six month of age was achieved. Subsequently, the triplet lambs born to this dam attained 12 month age in January 2010 with an amazing litter weight of 95.0 kg has been achieved. It clearly indicates that prolific sheep can be successfully reared / launched in the semi-arid region and it has great potential in augmenting mutton production in our country.



**Backcrosses- GMM / MGM:** Having developed prolific GM half-bred sheep, there was scope to exploit the prolific GM sheep for relatively better growth by backcrossing with native Malpura. Backcrossing of GM with Malpura was employed to make gain in body weights and growth performances and at the same time maintaining the prolificacy level comparable to GM sheep. In the backcrossing programme the *FecB* gene carrier GM rams were used as sires and Malpura ewes as dam breed to produce the GM x Malpura (GMM; 75% Malpura and 25% Garole) and reciprocal crosses were also attempted to produce MGM and their reproductive and productive performances were analyzed.

Percentage of multiple births ranged from 6.7 to 40 in GMM and from 14.3 to 27.3 in MGM. The litter size at birth ranged from 1.07 to 1.42 in GMM and 1.14 to 1.29 in MGM crosses. The incidence of multiple births is low compared to GM mainly due to lower *FecB* gene frequency in population and retention of more number of non-carrier females in flock. Once a sizeable population of GMM ewes are reached more rigorous selection for *FecB* gene will be carried which will further increase the prolificacy. The survivability of the back crosses (GMM/ MGM) is better than the GM and at par with the native Malpura sheep. The survivability for all age group over the year was >92% in GMM and > 95% in MGM, indicating better adaptability of GMM under prevailing climatic and managerial conditions. Least-squares means for body weights at birth, 3, 6 and 12 month were 2.54, 13.68, 20.00 and 26.15 and 2.13, 12.16, 16.59 and 21.10 kg, respectively in GMM and MGM crosses. The results indicate that there is pronounced higher gain in the body weight and growth rate of lambs produced after backcrossing of GM rams with Malpura ewes. This also reflects the major role of large uterine capacity of Malpura ewes in producing heavier GMM lambs compared to lighter MGM lambs borne by GM ewes. At present backcrossing is in progress to produce only GMM and production of MGM is stopped.



**Comparative performance of different prolific genotype with Malpura:** The ewe's productivity efficiency (EPE) is the best indicator of production performance of prolific sheep. The EPE at birth was highest in Malpura. From the weaning onward the EPE of GMM animals are higher than Malpura, mainly due to comparable growth with Malpura and high prolificacy. When comparison was made according to EPE recorded for GMM producing multiple births versus overall Malpura, there were 29.39, 27.59 and 31.0% improvement in EPE at weaning, 6 and 12 month weight, respectively. The GMM animals also scored over GM and MGM animals especially at market age of 6 and 12 month in terms of EPE. This is mainly attributed to comparatively higher body weight and better survivability leading to higher litter size

at various stages of growth. As of now incidence of multiple births is low in GMM compared to GM, mainly due to lower *FecB* gene frequency in population, retention of more number of non-carrier females in flock and parity of the females present in the flock.

**Introduction of Patanwadi inheritance for further improving the performance of prolific sheep:** Problem of low milk yield from the dam is still persisting in the 3/4<sup>th</sup> crosses (GMM/MGM), which is one of the major factor for lamb mortality, and if this problem is overcome by any intervention then EPE of prolific sheep can be further improved compared to present level. Hence, Patanwadi inheritance is proposed to be introduced in the GMM to increase the milk yield of dam. In this process, GMM and Patanwadi were crossed to produce 3 breed crosses which will result in higher prolificacy, higher birth weight along with more milk yield of dam to feed larger lamb crop per ewe. The breeding on the above proposed line has been started and initial findings are quite encouraging however the data size is very low to reach at any decision but these initial results have given a clear cut indication of scope of improvement in all production and reproduction traits. This intervention will meet the demand of farmers for more body weight and more number of lambs per lambing, resulting in higher economic returns. Screening and retention of carrier lambs and Inter-se mating and selection in different genetic groups will be followed to maintain the prolificacy as well as other desired traits. The new genotypes with traits of high prolificacy, more body weight and more milk per dam will be evaluated at the farm and field level. The improved and proven technology will be delivered at the farmers' door step to enhance his economic returns per unit of sheep for livelihood security.



**Kendrapada:** It is identified as second prolific sheep of India. A survey was made to study the prolificacy of Kendrapada sheep. In the flock surveyed more than 75% ewes produces multiple birth and the average body weights at milk, 2, 4, and 6 teeth were 5.57, 14.27, 18.86 and 21.42 kg, respectively. The Kendrapada sheep was purchased in month of November 2010 from its breeding tract. About 84% of animals were *FecB* gene carrier. The animals are under adaptation to the semi arid climate which is quiet different to conditions in their breeding tract

### Network project on sheep improvement

The Network project on sheep improvement (NWPSI) being on 1.4.1990, when all the centers of AICRP on sheep breeding were transformed into NWPSI Centers. AICRP on sheep breeding was mainly focused on crossbreeding of genetically low yielding indigenous sheep breeds with high yielding exotic sheep breeds viz. Rambouillet, Dorset, Suffolk etc., whereas, in NWPSI emphasis has been given on the survey, evaluation, conservation and improvement of indigenous sheep genetic resources. Under NWPSI different breeds of sheep are being improved through selection and inter-se mating for wool and mutton production. Presently, there are 10 cooperating centres of NWPSI (6 farm-based and 4 field-based) in the country with its coordinating unit at CSWRI, Avikanagar. The technical programme aims at improvement of indigenous sheep breeds under farm conditions wherein the male lambs are first ranked using selection index based on body weight and wool yield at six months of age. Best lambs are selected and mated at the age of 18 months. Subsequently these rams are again evaluated based on their progeny performance and best 2-3 rams are selected and used for breeding. Selected male lambs from improver flocks are supplied for breeding to base flocks.

### Mega sheep seed project

It was started on 1.4.2009 with the main objective to produce around 80 breeding rams of a breed every year and to cover about 8000 breeding ewes using selected rams by the end of XI plan. The project was sanctioned with

four participating units namely, KVAFSU, Bidar for Mandya Sheep, MPUAT Udaipur for Sonadi Sheep, TANUVAS, Chennai for Mecheri Sheep, BAU, Ranchi for Chottanagpuri Sheep and CSWRI, Avikanagar as Co-ordinating unit.

### AICRP on Sirohi goat

The unit of AICRP on Goats at Avikanagar came into existence in 1976. The objectives then laid down were to develop a new breed of goat suitable for agro-climatic conditions of Rajasthan and capable of attaining 25 kg live weight at 6 months of age with 45-50% dressing percentage under intensive feeding. It was envisaged in the technical program to cross the local Sirohi goats with exotic Anglo-Nubians and indigenous Beetal goats. But Anglo-Nubians could not be procured. The studies were, therefore, restricted to pure breeding of Beetal and Sirohi and crossbreeding of Sirohi with Beetal up to March 1984. The objective of attaining 25 kg live weight at 6 months of age was achieved. The crossbreds were, however, found only marginally superior to purebreds. The objective of the project was then modified to study the "Production performance of Sirohi, Beetal, Jakhrana, Marwari and Kutchi indigenous breeds" and the technical program was accordingly changed with effect from 1.4.1984. In 1986, the objective was again changed to compare the purebred performance of Sirohi, Marwari and Kutchi breeds for meat production under semi-intensive and intensive management systems and the Beetal and Jakhrana breeds were dropped from the technical program. Selective breeding of Sirohi is running as existing unit of AICRP at Avikanagar. Bucks used for breeding purpose were earlier selected based on 9 month weight and dam's 1<sup>st</sup> lactation 150 days milk yield. The index has been revised to include dam's 1<sup>st</sup> lactation 90 days milk yield in place of 150 days. Performance of Sirohi breed at farm for the year 2009-10 with regards to body weight at birth, 3, 6, 9 and 12 months of age were 3.22, 12.10, 19.68, 23.98 and 26.43 kg, respectively. The growth rate in terms of per day gain per head worked out to be 98.51 g from 0 to 3 months and 51.03 g for 3 to 12 months. The total milk yield in the does kidded averaged, 107.77 kg and average total lactation length was 174.56 days. The tugging percentage was 91.30. The overall mortality rate is very negligible. A total of 616 animals were sold to the farmers during last five years.



## Animal Nutrition

### A. Sahoo

The Sheep Nutrition Section was established in 1965 with the objectives to determine nutritive value of locally available feeds and fodder for sheep, to work out the nutrient requirements for maintenance, growth and production in sheep, to develop suitable feeding and grazing practices and to recommend efficient economic rations for sheep production. During the VI Five Year Plan the Division of Nutrition including Grassland and Forage Agronomy (GFA) was created to conduct research on various aspects of feed and fodder resource development for sheep and rabbits, the nutritive evaluation and performance of sheep and rabbits on various feeding systems and to develop feeding systems. At a later period GFA was separated. Since then Division of Animal Nutrition concentrated its efforts on:

- ❖ To evaluate various types of feed resources- pasture (grasses, legumes), top feed resources, agro-industrial by-products and non-conventional feeds
- ❖ To determine nutritional requirements for various production functions in sheep and rabbits
- ❖ To develop feeding strategies for sheep, goat and rabbit production
- ❖ To identify and reduce the adverse effect of various anti-nutritional factors in the feed
- ❖ To improve low grade roughages

Later on, the work on bioenergetics and rumen metabolism has also been taken up. The component-wise major research achievements of the Division are as under:

#### Feeds and feeding practice and nutritional intervention

The study on strategic supplementation of protein and energy for enhancing growth in Malpura sheep under field condition and socio-economic evaluation of nutritional interventions was carried out in Rajasthan. Primary data at farm gate level on the on-going feeding practices, socio-economic conditions and marketing pattern etc in sheep husbandry was collected through bench mark survey. The limiting nutrients viz., protein, energy and macro and micro minerals were priorities based on primary data pertaining to existing feeding practices. The benefit accrued from concentrate supplementation in term of weight and economic gain was documented and demonstrated to the farmers in the villages. The socio-economic impact of nutritional intervention in term financial gain to farmer was also demonstrated. To increase pre-weaning growth in lambs, concentrate mixture supplementation at the rate of 1.5% of body weight was demonstrated in the farmer flocks. The supplementation of lambs with concentrate mixture (@1.5% body weight) during pre-weaning stages improved weights by 24.18% and fetched 25-33% more price through sale of lambs in the market. Concentrate mixture at the rate of 1.5% of body weight was supplemented in lambs during post weaning stage. It resulted in increased body weights by 22-24% and lambs fetched 30-35% more price in the market. Planned supplementation of 300 g of concentrate mixture during pregnancy and lactation stages was demonstrated in field flocks. Use of cheaper mustard cake in place of costlier cotton seed cake was demonstrated in field. With the change in feeding of cheaper mustard cake in place of cotton seed cake, farmers are now saving Rs 90.00 per cow per month without compromise of milk production.



### Expanding feed resource base

On evaluation of the nutritive value of various feeds—grasses, legumes, top feeds and pastures available during different seasons, ardu leaves (top feed) were found to be the best in term of palatability and nutrient contents. Nutritive value and animal performance of artificially grown barley fodder (Fometa) was also assessed in sheep. The average yield of Fometa fodder was 10.4 q/day containing 86.95% moisture, 15.74% CP, 9.37% CF, 1.97 % EE and 70.27% NFE. The digestibility of DM and proximate principles except CF ranged between 75-90% whereas CF digestibility was 65%. The nutritive value of Fometa fodder assessed on hoggets indicated DCP and TDN content of 12.5 and 82.9%, respectively. The DMI of animals during sole Fometa feeding ranged between 3.2 to 4.0% of their body weight. The hoggets maintained on sole Fometa fodder consumed 840 g DM, 105 g DCP and 659 g TDN/day and gained at 34 g/day whereas the hoggets on Fometa fodder with cenchrus hay gained at a higher rate. It was thus concluded that the nutritive value of barley Fometa fodder was comparable to barley grain. When Khejri leaves (*Prosopis cineraria*) as dry or as fresh lopping were fed to sheep and goat, sheep lost 28 g/day weight whereas goats gained 46 g/day on a fresh lopping. When dried leaves were fed, sheep lost and goats just maintained their weight. Incorporation of khejri leaves in the ration of weaner lambs (20% by weight in the concentrate), lowered the digestibility of organic constituents. DCP and TDN values of dried khejri leaves were 4.6 and 31.6% for goats and 1.5 and 24.9% for sheep, respectively. Pala leaves (*Zizyphus nummularia*) had 5.6% DCP and 49.7% TDN with a nutritive ratio 1: 7.9. Dry matter intake from green pala leaves was 2.3% of body weight in growing lambs. The DCP and TDN values were 5.7 and 58.0%, respectively. A ration containing 30% Pala, 40% maize, 20% groundnut cake, 7% molasses, 2% mineral mixture and 1% common salt when fed to weaner lambs *ad libitum* for 90 days, they gained @ 100 g/day with 15% feed efficiency. Pala leaves should be fed in combination with other feed but not as a sole feed to obtain satisfactory performance. Treatment of pala leaves with 1.2% PEG (Polyethylene glycol- 4000) significantly increased dry matter intake and dry matter digestibility. The body weight increase in yearling rams was also higher in PEG treated group than untreated group. The dry leaves of Mopane (*Colophospermum mopane*) had poor preference in the sheep while green leaves and twigs were consumed. Karakul crossbred adult animals could consume the dry matter at 2.3% of body weight from green leaves and chaffed sewan hay and maintained their weight. The ground ripe Siris (*Albezzia lebbeck*) pods could be mixed in the concentrate mixture for adult Marwari sheep to the level of 40% without any deleterious effect on health and wool production. The groundnut fodder is very good for sheep as it contains 6.4% DCP, 59.07% TDN with net return as 1: 8.1. Prickly pear cactus or opuntia, [*Opuntia ficus-indica* (Linn.) Mill.] from semi-arid regions in India contain CP, 92-126 g/kg DM, which is higher than the commonly used dry roughages (straw, stover and grasses) in ruminant feeding. Although opuntia feeding with conserved fodder maintained adult sheep, a high N loss in urine is the principal concern and may require additional N supplementation. Sheep offered Opuntia in combination with cenchrus (*Cenchrus ciliaris*) hay had low feed intake, apparent digestibility of DM, CP and energy, nutritive value, plane of nutrition and N balance, but when supplemented with 50 g groundnut meal, there was improvement and values were similar to cenchrus plus 200 g concentrate supplemented diet. Fallen tree leaves can be incorporated in the diet of sheep up to 20% in the feed block. Feeding trials revealed that oven dried leaves of *P. juliflora* can replace guar straw up to 20% whereas pods could replace up to 50% of guar straw. Sun dried ground pods of *P. juliflora* can replace 30 - 40% concentrate mixture fed to rams @ 1% of their body weight along with cenchrus hay fed *ad libitum*. Replacement of concentrate with dried pods of *P. juliflora* did not affect the nutrient intake, rumen fermentation characteristics and nutrient utilization. Dried *P. juliflora*



leaves can replace concentrate mixture, guar straw and cenchrus straw up to 10-20%. However, dried pods can replace concentrate mixture, guar straw and cenchrus straw up to 40-50%.

### Nutrient requirement studies

Requirements of nutrients (energy, protein) for sheep, mainly depends on body weight of animal, its interaction with environment, and level of different production functions. The body weight gain of crossbred hogget on a ration with 65% TDN and 8% DCP was 140 g/day. TDN and DCP requirement of hogget weighing 20 kg and gaining at 140 g/day was 617 and 65 g, respectively. Lambs weighing 15 kg consumed 889 g DM, 457 g TDN and gained 89 g/day. Lambs (25 kg weight) consumed 990 DM, 675 g TDN and 74 g DCP/day and gained 95 g/day. Consumption of TDN and DCP in the ratio of 8.5 at an intake level of 3 g DCP/kg live weight produced the highest growth in weaner lambs. Dry matter consumption was 3.5 to 4.0 kg/100 kg body weight. Fine wool crossbred lambs averaging 15 kg body weight consumed 617 g DM, 422 g TDN and 48 g DCP and gained 70 g/day. Avikalin lambs gained 151 g/day when fed a complete feed containing 50% concentrate and 50% roughage (ground cowpea hay) with an intake level of 1100 g DM, 642 g TDN and 156 DCP. DM intake worked out to be 46 g/kg body weight in lambs. Thirty six grams of TDN and 5 g of DCP per kg metabolic weight have been recommended as the requirement for maintenance. Rams weighing 32 kg maintained weight with intake of 397 g TDN and 34 g DCP/day. For maintenance, approximately 10 g of TDN and 1 g of DCP are required per kg live weight. For good growth of lambs about 3 g DCP is required per kg live weight and TDN requirement is about 8.5 times the total DCP required. For young lambs, more than 14% protein in the diet had no additional advantage. Pregnant ewes during their advanced pregnancy required 7.8 g of DCP and 58.7 g of TDN per kg metabolic weight for producing lambs with 3.5-4.0 kg birth weight. These lambs attained 14.0 kg body weight at two months of age. Nutrient requirements of sheep at various physiological stages were estimated as under:

Attributes	Requirement/kgW <sup>0.75</sup>			
	TDN (g)	DE (kcal)	ME (kcal)	Digestible protein (g)
Maintenance	27.30	120.12	98.50	3.0
Growth (per g gain)	1.79	7.87	6.46	0.2
Pregnancy	50.90	223.96	183.65	7.0
Lactation	53.00	233.20	191.22	8.5
Wool production	35.00	154.00	126.28	4.2

The study on measurement of energy expenditure in sheep indicated that a housed sheep spent 4.16 MJ energy / day. The energy expenditure of grazing animals was 43% higher than that of stall fed sheep. The expenditure of energy was 4.85 MJ/ day during winter, which increased to 6.70 MJ/day during rainy season. The animals exposed to heat stress required more energy to meet their enhanced requirements for thermoregulation and maintenance. The extra requirement by suitably increasing the energy density of the diet improved production traits of weaner lambs and adult sheep. Optimum nitrogen-sulphur ratio in the diet of wool producing sheep was found to be 5:1. Sulphur concentration of 0.24% in the diet produced maximum quantity of wool i.e. 1392 g in 6 months clip with a scouring yield of 80%. On increasing the feeding level by adding 20% concentrate of the cenchrus fodder, wool production was increased from 671 g to 1393 g and staple length was increased from 3.7 to 5.6 cm. The effect of dietary levels (18, 22 and 27%) of crude protein on pre-weaning gain in lambs indicated that even the lowest protein level was optimum for their pre-weaning growth requirement and the weaning weights were 11.1, 11.3 and 11.4 kg on diets with 18, 22 and 27% CP, respectively. These lambs, on intensive feeding, on a diet containing pala (50%) and concentrate (50%) had similar finishing weight of 21 kg. Maintenance requirement of protein in adult Marwari rams weighing 30 kg was 6% CP. The optimum level of CP and TDN in the ration for wool production was 10 and 50%, respectively. On the basis of

nutrient availability and requirement and possible supplementary feeding a strategic supplementary schedule is developed to have optimum production from flock, viz. 330 g ground maize to dry non-producing animals (30 kg) only during summer, maize + oil cakes at 300+100 g, 0+115 g and 240+200 g to pregnant (30 kg) and 0+125 g, 0+190 g and 220+250 g to lactating animals during monsoon, winter and summer, respectively.

### Nutrition of sheep on pasture

The main pasture species was *Cenchrus ciliaris* and the grass legume mixed pasture has cenchrus and dolichos. Cenchrus - dolichos mixed pasture could produce growth at 45 to 100 g / day in lambs. The CP% in the native pasture varied from 4.3 to 10.3% and remained below 7.0% during September to March. DM and CP contents of pasture during lush and lean seasons were 31.5, 8.0% and 76.2, 4.5%, respectively. TDN intakes by rams on available pasture were low (20 to 50% of NRC allowances). DCP intakes were adequate during July to September only. Phosphorus intakes were also not adequate for the most part of the year. Vitamin A level declined gradually from September to December and June. A stocking rate of 5 ewes/ha on cenchrus gave satisfactory performance of Avikalin and Avivastra ewes. When ewes were allowed to graze on such pasture at a stocking rate of 5, 6 and 7 sheep/ha, forage availability during July was reduced to 5.1, 4.5 and 1.1 q/ha, suggesting that 6 sheep could be carried per hectare on cenchrus pasture. Mutton synthetic sheep grazing 8 hr/day on cenchrus dominant pasture performed well with annual lambing 83.5%, adult mortality 5.5% and lamb mortality 6.5% and the body weight of lambs at birth, weaning (3 months) and at slaughter (9 months) was 3.0, 11.5 and 20.0 kg, respectively with dressing percentage of 40. Wool yield was 2.1 kg/annum/sheep. Dorset x Sonadi cross-bred ewes at the stocking rate of 6 ewes/ha performed well in terms of growth in cenchrus based silvi-pasture. The average body weights and lambing% of ewes were higher with stocking rate of 5 ewes/ha, however, average wool production in ewes were higher with stocking rate of 6 ewes/ha. Six month body weight was higher with stocking density of 4 ewes/ha. Cenchrus produced maximum fodder under more stocking rate (6 sheep/ha) due to maximum plant height as sheep dropping organic carbon content of surface soil increased and soil pH reduced. Avivastra ewes and Marwari does at stocking density of 4 animals/ha (2 ewes + 2 goats) grazing on *Cenchrus ciliaris* pasture consumed 37.2 and 28.5 g DM/kg body weight and DM digestibility of 60.8 and 51.0% during lush season and they consumed 28.5 and 27.3 g DM/kg body weight with DM digestibility of 45.3 and 40.3% during lean season. Birth, weaning (3 months) and 6 monthly weights of grazing lambs and kids were 3.3 and 3.2; 18.5 and 16.3 and 27.5 and 26.6 kg, respectively. After one year of grazing goats gained better (8.15 kg) than sheep (6.2 kg). Six monthly greasy fleece yields of the ewes ranged between 1.29 - 1.46 kg and in does the average daily milk yield ranged between 514-700 ml. In male hoggets grazing on three, two and single tier silvi-pasture plots and under routine sector management (the biomass availability in these pasture ranged from 21.72 - 28.91 q/ha) the live weight gain was higher in the silvi pastoral plots (7.3 kg) compared to sector management (3.4 kg). However, wool yield (six monthly clips) of these animals was similar (913 g). Nutritional studies at north temperate region revealed that body weight gain in crossbred (Rambouillet x Gaddi) hoggets was highest with white clover (*Trifolium repense*; 4.19 kg) and lowest with native grass ((*Cymbopogon* family - locally known as Kanja; 2.30 kg) and intermediate with Tall fescue (*Festuca arundinacea*). Finishing weight of 20-22 kg was achieved with ADG of 53-61g/day in lambs and 93-102 g/day in kids. Grazing sheep (Malpura and Mutton Synthetic) revealed that silvipastoral system of range management could successfully maintain 4 ewes with followers round the year. However, to obtain the desired production traits the surplus biomass available on pasture had to be harvested to avoid losses in natural cycle and fed to the animals during lean season and critical physiological stages. Fallen biomass formed the major portion of diet during summer months and its preference by the grazing animals increased from February (30.0%) to March (47.3%). Grazing sheep found to prefer *Eluecina indica*, *Commenlina forsakalaei*, *Eriochloa polystachyca* and *Crotalaria burhia* during onset of monsoon. These were found to be nutritious, thus need protection during bush cleaning operations. Marwari ewes could be maintained on pasture grazing alone throughout the year when not bred. Dry matter intake was highest during October to December, but digestibility of nutrients was



the lowest during this season. Digestibility of nutrients was significantly reduced during advanced pregnancy as compared to early pregnancy and lactation. There was need for additional supplementation of energy and protein during advance pregnancy and lactation for better lamb production. Marwari lambs gained 131 g/day during the pre-weaning period and attained weight of 15 kg at 3 months. Overall average concentrate consumption during pre-weaning was 148 g/day. They could attain body weight of 35kg at one year of age under adequate feeding. Body weight gain of sheep in one year period was better on improved pasture (7- 8kg) compared to natural (5.6 kg), improved natural (5.4kg) and reseeded cenchrus pasture (6.1 kg). In goats, however, gain in weight was higher in reseeded cenchrus pasture (8.4kg) compared to natural (8.3), improved natural (8.2kg) and improved (7.9kg) pasture. Average weight at 3 months of age was 19.4kg whereas kids' weight at 3 months of age was 17.6kg. Performance of kids was better than lambs when grazed on silvi-pasture system. While estimating DM and DE intakes using different diet collection methods e.g. composite pasture sampling (CPS), mouth grab (MG), hand plucked (HP) and oesophageal extrusa (OE), and faecal collection methods e.g. use of collection bags and chromic oxide indicator, it was observed that, the CPS, HP and MG methods over estimate DM and DE intake. The OE method, however, had limitation of salivary nitrogen contamination, alteration in grazing behaviour due to surgical trauma and artifact lignin formation in drying process. The double indicator method has been successfully modified to reduce errors. In the process oesophageal cannula was also fabricated and fitted in sheep for collection of representative sample of diet of grazing animals. The DMI estimated by extrusa and total faecal collection was 665 g/head/day and was significantly lower than that estimated by composite (1265 g/head/day), mouth grab (847 g/head/day) and handpicked (1095 g/head/day) method. Nutrient selectivity studies using oesophageal cannulated sheep, suggested that animal could preferentially select moderately high protein (CP 13%) diet, even during lean season, when pasture contained about 3-4% CP.

The biomass yield of community grazing land in the month of September, January and May was 4.92, 1.36 and 1.93 DM q/ha. Most of the native grass species were sprouted immediately after rain and constituted the pasture for grazing by sheep viz. *Tribulus terrestris* (8.90%), *Indigofera cardifolia* (16.24%), *Crotolaria burhia* (12.64%), *Satha* (16.91%), *Zizyphus nummularia* (11.40%), *Dactyloctenium aegypticum* (21.18%), *Melilotus indica* (9.31%) and other native grasses, during winter *Crotolaria burhia*, *Zizyphus nummularia*, dead litter and *Azardirachta indica* leaves at 9.75, 40.34, 24.61 and 23.94% and in summer *Prosopis cineraria* (33.32%), *Cynodon dactylon* (28.03%) and *Zizyphus nummularia* (12.19%). Sheep diet contained DM 56.18%, CP 13.90%, NDF 56.90%, ADF 34.76%, lignin 8.36% and total ash 20.90% during monsoon and then DM increased and CP decreased in the following seasons. The nutrient intake and digestibility in dry, pregnant and lactating sheep were significantly higher during winter in all the three physiological stages as compared to monsoon and summer. Average digestible crude protein (DCP) intake of sheep was 54.7, 43.4 and 13.0 g/day during monsoon, winter and summer season and was 34.7, 32.3 and 44.1 g/day in dry, pregnancy and lactation stages and the corresponding ME intake (MJ/day) was 8.90, 10.30 and 4.51 and 7.32, 7.26 and 9.15. Soil ingestion from plants, as plant's contaminants and ground in sheep was 92, 52 and 162g/day during monsoon, 53, nil and 83 g/day during winter and 64, 43 and 230g/day during summer respectively. Total soil ingestion during monsoon, winter and summer was 360, 137 and 337g/day and 26.91, 11.97 and 41.04 g % of DMI. The intake of DM ranged from 672 to 1290 g in dry ewes depending upon the availability of pasture and nature of vegetation. The intake of DM OM, CP, ME were similar in dry, pregnant and lactating ewes but DCP intake was significantly ( $P < 0.05$ ) lower in pregnant and lactating ewes vis-à-vis dry ewes. The intake of DM was 60.5 g higher in dry ewes and 696.3 g lower in pregnant ewes as compared to the ICAR (1998) requirements. The intake of DCP was more (33.0 g) in dry ewes and lower (49.1 g) in pregnant ewes vis-à-vis ICAR standard. But the energy intakes in both the stages were higher than ICAR standard for similar body weight and physiological stages. Pasture yield of cenchrus (q DM/ha) was higher in the monsoon (40.7) than October (24.6) and December (10.8). The DM was higher in December (82.9%) and lowest in August (46.5%) with reverse trend in CP showing the lowest value (6.01%) in December. The fibre composition was in line with DM. The concentration of CP was higher ( $P < 0.001$ ) during August (9.40%) and lower during June (7.54%). The chemical composition of other components namely, NDF and ADF

content of the pasture was higher in June and lower in August. With a DM intake of 737, 656 and 681 g/d in the month August, October and December, respectively, the digestibility of DM, OM, CP, NDF and ADF of ewes were high in August (54.7, 54.1, 60.6, 56.5 and 59.3%) and low in December (50.8, 50.2, 47.7, 21.3 and 34.6%). The digestible crude protein (DCP %) also varied among seasons ranging from 5.72% in December, 6.61% in October to 8.48% in August. The ME intake during August, October and December were 35.1, 22.6 and 35.5 MJ/d, respectively that supported higher live weight gain in December versus August and October. The biomass yield of community grazing area and fellow land was 6.2 q DM/ha. Pasture sample collected from community grazing land mainly comprise of Chidichawla, Bekariya, Doodhi, congress grass and Doob. Four major contributors were Doodhi (32.6%), followed by Bekaria (19.4%), Chidi chawla (15.71%) and Congress grass (12.15%). DM, CP, NDF, cellulose and lignin content of the pasture sample was 52.55, 9.37, 55.57, 26.22 and 6.80 respectively. The CP content of collected individual vegetation samples ranges from 11.38-13.89% and lignin 4-7%. The CP content of the diet sample was quit high 12.77% and it was 26.62% higher than pasture samples. Preference index for DM and CP was 0.66 and 1.32, respectively indicating animals prefer to consume succulent material from pasture having high nutritive value.

### Supplementary feeding

Grazing alone could hardly produce satisfactory growth in lambs. Supplementation with concentrate in addition to 8 hr/day grazing on cenchrus dominant pasture increased carcass yield by 55%. Crossbred weaner lambs on an average getting 600g concentrate per day in addition to grazing gained @ 130 g/day compared to 108 g/day on 50% concentrate and 50% roughage diet in stalls. Supplementation of 200 g concentrate or cowpea hay produced weight gain of 70 g/day. Supplementary feeding, (concentrate, hay and green) over grazing to kids, resulted in an increase of 45% in live weight and 14% in dressing percentage. A daily gain of 37, 87 and 73 g from 3 to 6 months and 63, 139 and 120 g from 6 to 9 months age was obtained in Beetal X Sirohi kids maintained on browsing, browsing with concentrate supplementation and on complete stall feeding, respectively. Fine wool crossbred lambs (about 10 weeks of age), grazing- on cenchrus pasture were given concentrate (*ad libitum*, and 75%, 50% and 25% of the *ad libitum*) for 194 days. The lambs in above four groups consumed on an average 656, 490, 327 and 163 g, concentrate daily and gained 123, 110, 104 and 98 g/day respectively, as compared to 67 g/day by the group on grazing alone. At 280 days of age, the lambs attained body weight of 35, 33, 32, 28 and 24 kg in 5 groups, respectively. Average wool production was 1249, 1218, 876 863 and 616 g per animal in 5 respective groups. Average milk yield (ml/day) of ewes in three, two and single tier silvi-pasture and natural rangeland were 467, 365, 310 and 285 respectively with milk protein content of 5.0 - 5.4 g %. Supplementation of formalin treated GN cake (1% v/w) did not produce any significant effect on overall weight gain of lambs but wool production increased by about 10%. The CP digestibility decreased in the experimental group. Staple length, fibre diameter and crimpiness were not affected whereas there was a non/significant reduction of fibre density by formaldehyde treatment.

In Kutchi goat breed, milk yield in the does averaged 63.84 kg for 90 days and 78.71 kg for 150 days. The means for the live weights pooled over sexes at birth and 3 months of age were 2.80 and 11.04 kg, respectively. The respective values for males were 2.91 and 11.12 kg and for females 2.69 and 10.96 kg. The growth rate pooled over was 91 g/day during 0 - 3 months age. When groundnut cake untreated or treated with 1 % formalin for one hour and dried at 80° C was offered to rumen fistulated rams along with pearl millet stover *ad libitum* and mineral licks there was a marked decrease in rumen ammonia-N concentration on feeding the formaldehyde treated cake than on untreated cake. The TVFA and pH levels did not show any difference between the groups. Experiment on weaner lambs for mutton production under intensive feeding management and supplementary feeding along with grazing to study growth rate and nutrient utilization indicated that under both the systems of feeding lambs achieved 23 kg body weight. The average daily gain (g) 98 and 106 and feed efficiency (kg/kg gain) 11.2 and 12.0 with DMI (g/kg W0.75) 100.6 and 92.9 was observed under intensive and supplementary feeding systems, respectively. At maintenance

level of feeding (roughage to concentrate ratio, 65:35), the rumen pH, TVFA and TCA soluble nitrogen were same in sheep and goats whereas the concentration of total nitrogen in rumen liquor was higher in goats. Concentration of ammonia-nitrogen was higher in sheep than goats. Total protozoa population was similar in both the species ( $3 - 4 \times 10^5$ /ml SRL). Total number of holotrichs and large spirotrichs were almost similar in both the species but the number of small spirotrichs was higher in goats than in sheep. Sorghum incorporated in lamb ration, was as good as barley. Spent wheat (broken, leftover in Godowns, not fungal contaminated) can also be incorporated in high-grain lamb ration.

Groundnut cake in sheep ration can be effectively replaced by cotton seed cake on equal nitrogen basis. Addition of cotton seed cake at lower levels (up to 20%) did not affect body weight gains in lambs. Guar korma, a locally available agro-industrial by product may be mixed as a protein source in the ration of adult sheep in place of cake without any adverse effect on wool production and health while it may replace groundnut cake nitrogen in the concentrate mixture for Marwari hoggets to the level of 35% without any adverse effect on weight gain, wool production and health. Dietary inclusion of damaged wheat by replacing maize at 75 and 100% levels depressed growth although serum IgG level was unaltered indicative of no adverse effect on immunity.

### Improving mutton and wool production

Mutton synthetic (MS), Malpura selected (1M) and Sonadi (S) weaner (90 days) lambs could achieve 170, 150 and 118 g ADG with 1:5 to 1:7 feed efficiency ratio under intensive feeding on 50:50 roughage (pala leaves) and concentrate based ration. In intensive feeding out of 99 MS lambs used, 90 per cent animals achieved finishing weight of 30 kg in record time of 56 days (total age of 146 days) and out of seven Malpura lambs 71% achieved 30 kg live weight in 66 days (total age of 156 days). Weaner lambs (Avivastra) and kids (Marwari) were provided intensive feeding with composite ration having 50:50 roughages and concentrate. In 90 days of intensive feeding, the weaner lambs (19.2 kg) and kids (17.2 kg) achieved finishing weight of 35.9 and 23.0 kg with ADG of 185.0 and 64.0 g and feed efficiency ratio 1:8.9 and 1:14.3, respectively. Nali, Chokla, and their crosses with Merino/Rambouillet weaner (90 days) lambs maintained on 50:50 roughage concentrate based ration achieved average daily gain of 111 to 135 g with 1: 12-15 feed efficiency ratio. The mutton synthetic male lambs maintained on creep ration up to 60 days, on 70:30 concentrate: roughage ration from 60 to 100 days and on a 50:50 ration for 100-130 days reached a live weight of 30 kg with a daily gain of 204 g/ head. These lambs consumed 11.5, 9.3 and 8.0 g DCP and 55, 56.4 and 57.5 g TDN/kg  $W^{0.75}$  day at average body weights of 16.6, 23.1 and 30.5 kg, respectively. Body weight of 20-22 kg in indigenous and 24-25 kg in cross breeds was achieved by supplementary feeding of creep ration (150 and 200 g/day) in addition to suckling up to weaning and 250 to 350 g/day concentrate mixture in addition to grazing up to 6 months of age. To avoid higher deposition of carcass fat and to improve the efficiency of feed conversion the lambs were weaned at 60th day of age and intensively fed to achieve target finishing weight of 25 kg. The MS, 1M and MC lambs achieved finishing weight of 25 kg in 73, 91 and 136 days of intensive feeding with 162, 135 and 112 g ADG and 18, 16 and 14 % feed efficiency, respectively. To sustain the gain the lambs consumed 75 g DM, 5.5 g DCP and 42.1 g TDN/kg  $W^{0.75}$ . Experiments with lambs fed on various levels of digestible energy and protein indicated that they required 70 g DM, 5 g DCP and 50 g TDN/kg  $W^{0.75}$  to support 100g ADG.

Supplements, maize, barley, jowar, bajra and damaged wheat in feedlot rations of weaner lambs indicated that all these energy sources could be effectively utilized in growing animals to achieve ADG ranged between 92-114 g in different groups. In Mutton Synthetic lambs, growth responses were better with groundnut and cotton seed cake (137 g ADG) compared to guar meal, mustard cake and urea replacement (112 g ADG) of the cakes. The lambs required 70-80 g DM, 5-6 g DCP and 40-50 g TDN/kg  $W^{0.75}$  to support 100 g ADG. Experiment was conducted on newborn Malpura lambs (15 day old) under three feeding regimen, group G1 was on conventional management

practices viz., milk suckling from the ewes and pasture grazing plus green tree leaves during night, group G2 with additional *ad libitum* creep mixture, group G3 with *ad libitum* milk replacer and *ad libitum* creep mixture. The weaning weights were significantly higher in G2 and G3 group as compared to G1 showing average daily gain 89.6, 146.6 g and 154.4 with feed conversion ratio 4.60, 3.86 and 3.81 in G1, G2 and G3 groups respectively. The body weights after six months were 27.2, 31.1 and 32.0 kg in G1, G2 and G3 group respectively with no significant post-weaning gain but a relative increase in wool yield and staple length in G3 followed by G2 and G1 proportional to higher live weights of animals. However, the feed conversion ratio was better in G2 (5.13) and G3 (5.17) compared to G1 (6.32). Fat supplementation up to 5% increased weight gain, improved feed conversion ratio and maintained the carcass quality within acceptable norms. It reduced feeding cost per kg weight gain and improved benefit: input ratio at 5% fat inclusion level for mutton production. No effect of fat supplementation was observed on carcass traits and composition. The economic analysis revealed advantage of rumen protected fat supplementation in the diet and a level of 2% gave higher returns as compared to 4% level. In cull ewes concentrate feeding @ 2.5% of body weight up to 90 days improved body conditions and there is improvement in weight gain from 24-30% of initial weight. The dressing yield improved by 7-8% bone content in carcass decreased by 5-7% with fat content below 20%. Decreasing the feeding period below 90 days with concentrate feeding level beyond @ 2.5% of body weight did not yield any advantage in input output ratio in cull ewe. Replacement of conventional protein cakes with non protein nitrogen sources did not yield extra advantage as the economic analysis revealed higher profit in conventional cake diet as compared to urea supplemented diet. Rumen bypass fat supplementation in the ration of cull ewes increased weight gain from 20.35% in control to 25.66% with 2% and 30.95% with 4% level. There was positive carcass attributes with better cost: benefit ratio with 4 % rumen by pass fat supplementation as compared to other levels as well as control. In lamb ration when the ration was supplemented with different coconut oil the digestibility of DM and OM increased up to 2.5% level of fat supplementation after which it decreased. Fat supplementation up to 5% level increased weight gain, improved feed conversion ratio, maintained the carcass quality within acceptable norms, reduced feeding cost/kg weight gain and improved benefit: input ratio is advantageous in mutton production. The rumen bypass fat supplementation revealed effect during post weaning period as the six month body weights increased linearly with fat level up to 4% level. Average daily gain and feed conversion ratio and digestibility of dry matter improved with rumen by fat supplementation. The economic analysis revealed advantage of rumen protected fat supplementation in the diet and a level of 2% gave higher returns as compared to 4% level. Carcass and wool quality improvement in lambs was tried with supplemental protected fat vitamin E and positive response was obtained in both bypass fat and vitamin E supplemented groups. Khejri leaves incorporated as a source of polyphenols to improve quality parameters but a relatively lower feed intake subsidised any such improvement. For wool production it was revealed that sulphur supplementation @ 0.3% in the concentrate containing either low protein or high protein did not improve performance or wool quality parameters. However, an increase in wool yield (around 450 g) with a reasonable increase in fibre diameter and decrease in hairy and medullation percentage signified positive effect (good carpet wool type) of sulphur (0.3% S as  $\text{Na}_2\text{SO}_4$ ) supplementation in sheep on grazing plus concentrate ration.



### Assessment of methane emission and mitigation strategies

Among cereals methane emission per 100 mg of digestible organic matter was lower in pearl millet (1.41 ml) and higher in guar grains (1.83 ml) whereas in cakes it was higher in cotton seed (1.72 ml) and lower in mustard cake (0.56 ml) and in by product the values were higher (2.04 ml) as compared to grains and cakes. Methane production

per g of samples was highest in the leaves of kala siras (13.99 ml) followed by shisham (10.44ml) and siras (9.70 ml) leaves. The tree leaves producing less methane were of khejri (2.27 ml), mango (3.58 ml), tamarind (3.83 ml) and sagwan (3.86 ml). Methane emission per 100 mg of digestible organic matter was higher in jamun leaves (2.94) and lower in tamarind leaves (0.62). In khejri leaves the value was 1.21 ml. Total methane production per g of sample in available crop straws in descending order was as til straw (12.25 ml), jowar straw (9.79 ml), wheat straw (7.86 ml), barley straw (7.79 ml), saunf straw (7.33 ml), guar straw (6.55 ml), gram straw (6.20 ml), groundnut straw (5.38 ml), jeera straw (4.19 ml) and bajra straw (2.21 ml). Methane emission per 100 mg of digestible organic matter was higher in barley and lower in til straw. Methane emission reduced with the level of supplementation in both the lamb as well as adult ration. Methane emission per 100 mg of digestible organic matter was reduced with bypass supplementation in both the diet. Among the spices straw, Ajowin, fenugreek and fennel produced lower methane up to 15% level of inclusion. The selected spices straws (at 5, 10 or 15%) were tested in complete diet with different concentrate and roughage ratio (60:40 or 40:60 or 25:75 or 75:25) *in vitro*. Amongst the tree leaves, Khejri, Neem and Ardu up to 20% level of inclusion lowered methane production and improved digestibility to a significant extent. It is found that inclusion up to 20% level showed reduced methane production with improved fermentation attributes and digestibility *in vitro*.

### Rumen metabolism, microbiology and biotechnology

Under control feeding, dry-matter intake was lower while digestibility of fibre fraction was higher in goats compared to that of sheep, which could be partly due to the greater number of total ciliate protozoa as well as holotrichs and spirotrichs in the rumen medium of goats. Sodium lauryl sulfate can effectively defaunate the animal and as a defaunating agent it had no apparent adverse effect on the performance of sheep as evident from similar nutrient digestibility, plane of nutrition, rumen fermentation pattern and ciliate protozoal population between refaunated and faunated sheep. A reduction in methane emission results in more ME availability for growth and wool production with a better FCR. With similar dry matter intake and plane of nutrition, the growth performance and feed conversion efficiency were better in defaunated than refaunated and faunated lambs. In spite of reduced digestibility of neutral detergent fibre, acid detergent fibre and cellulose, the ME availability for body synthetic activity was probably higher in defaunated lambs as evident from an enhanced production traits. A simplified cultural test using Fe-2 medium to detect mimosine degradation by the mixed rumen microbes of sheep and goat was performed. Mixed rumen microbes of sheep and goat degraded mimosine at 19 and 26%, respectively on 30<sup>th</sup> day of incubation. The influence of live yeast culture namely *Saccharomyces cerevisiae* (SC), *S. uvarum* (SU) and *Kluyveromyces marxianus* (KM) and a mixed yeast culture of above three in a ratio of 1:1:1 was assessed in weaner Malpura lambs. Among the three yeast strains, lambs fed yeast culture *S. cerevisiae* had significantly better performance in terms of total gain and ADG followed by mixed yeast culture and *S. uvarum* culture. Yeast culture feeding improved growth performance by 5 to 8%, feed efficiency by 3 to 9% and 6-12 reduction in cost of feed inputs per kg live weight gain. Fermented yeast feeding (@ 30-50 g) also reduced incidence and duration of diarrhoea in lambs. Feeding of fermented yeast product as probiotic supplement fermented product dried under shed at 20 to 26°C for 60 to 72 hrs had viable live yeast cell production of 4-14 x 10<sup>6</sup> per gram. This level of yeast cell viability was maintained at room temperature of 30-35 °C and can be used for animal feeding as and when required. Optimum dose of probiotics for better growth, health and mutton production was assessed in pre-weaner lambs. The lambs were drenched with liquid culture of *L. acidophilus* containing 3.6x10<sup>9</sup> cells/ml at 0 (G-1), 1 (G-2), 1.5 (G-3) and 2 (G-4) ml/kg body weight individually. Lambs in G-2 gained (1.7%) more weight than the control (G-1), while lambs in G-3 and G-4 showed lower weight gain. Average daily gain was better (150.4 g) in G-2 than the control. The intake of concentrate was higher in control group than probiotics fed group, while the intake of roughage was higher in probiotics fed groups than the control. The intake of



DM was higher in control and G-2 group and lower in G-3 and G-4 groups. Feed conversion efficiency was also similar in all the groups. With *Lactobacillus acidophilus* feeding faecal *E. coli* count was also lower in probiotics supplemented groups than the control. The incidence of diarrhoea was lowered and faecal consistency improved in *L. acidophilus* supplemented groups. Ruminants harbour a diverse microbial population in their fore stomach, rumen and the diet of the grazing ruminants consists of mostly poor quality roughages and contains higher structural polymers (cellulose, hemicellulose and pectin). Because the feed they eat, such as grass, leaves, and hay is not very nutritious, they have to get the most out of what they eat. The major species involved in cellulose degradation are *Bacteroides succinogenes*, *Ruminococcus albus*, *R. flavefaciens* and *Eubacterium cellulosolvens*. These bacteria closely adhere to the plant cell-wall surfaces forming erosion pits as they degrade cellulosic substrate. The enzyme responsible for cellulose degradation, cellulase is present only in rumen microorganism. The microbial population in the rumen is diet dependent and changes with the type of diet. However in grazing ruminants the microbes have better efficiency in utilizing cellulosic feed. A study was conducted to isolate and characterize cellulose degrading bacteria from rumen liquor of grazing sheep and also to identify the organism with the help of molecular techniques. Eight different bacterial colonies were separated and the different isolates were morphological characterized on the basis of different colour and size of their colonies, and then the isolates were biochemically characterized. The isolates showed varied characteristics with respect to biochemical characterization as stated above. All the isolates are Gm-ve but they varied morphologically from cocci to bacillus or coco-bacillus. The motile isolates were R1, R2, R5, R7 and R8. The gas and H<sub>2</sub>S production was positive for all the isolates except R6 and R7. The sugar utilization revealed cellobiose negative for isolate 7 and 8. In all other isolates, positive sugar utilization for cellobiose and glucose resulted in drop in pH and increase in absorbance indicative of growth of organisms in the media. Catalase test was negative for R1 and R3. Gelatin liquification test was negative for R4, R6 and R7. The anaerobiosis test revealed that all the isolates were obligatory except R1 which was facultative. The mono cultures were preserved at -20°C as bank and maintained for inventorization.

### Micronutrient profile and supplementation strategies

The results of AICRP on improvement of feed resources and nutrient utilization in raising animal production under existing feeding practices and gaps in feeding systems for livestock of different agro-climatic zones of Rajasthan has been documented. The micronutrient profile of macro (Ca, P and Mg) and micro minerals (Cu, Fe, Zn and Mn) and intakes of cattle, buffaloes, sheep and goats in different physiological stages have been worked out from the actual mineral contents of feeds and fodders fed to animals in different agro-climatic zones and compared with standard requirements for identifying the mineral deficiencies. The prioritization of these nutrients in livestock feeding has been identified and a suitable strategy for their supplementation has been developed.



Area specific mineral mixture consisting of calcium, phosphorus, zinc, copper and cobalt minerals in different proportions for different agro-climatic zones of semi-arid Rajasthan has been developed based on deficiencies of these minerals in the diets or rations of animals during different physiological stages. Mineral mixture pellets were prepared for supplementation to sheep and goats. The supplementation of mineral mixture at the rate of 40g daily in cattle during lactation stage increase milk yield by 10-15%, bring the cows into estrus within 30-45 days of supplementation and ease the problem of skin keratinization. The supplementation of mineral mixture provides additional net income of Rs 216.0 per

animal per month through sale of milk. It has been found that mineral mixture helps in bringing anoestrus sheep into estrus after 15-20 days of supplementation. The supplementation of mineral mixture at the rate of 5g daily for 5 month increased the wool yield from 400g in non-supplemented pregnant sheep to 433g in supplemented lactating and 440g in supplemented dry sheep. Chelated copper and zinc in place of inorganic minerals used in mineral mixture for supplementing to Malpura lambs under field conditions. Growth of lambs on roughage and supplementary concentrate feed containing conventional and chelated copper and zinc minerals were almost similar however copper and zinc in chelated form needed 56.38 and 25.90% smaller in quantities than conventional inorganic source to achieve same level of growth in lambs.

### Improvement of low grade roughage

Ammonia-sulphuric acid treatment of wheat bhusa increased dry matter digestibility in sheep. Adult animals receiving *ad lib* pearl millet in the stalls was provided licks containing biuret and mineral mixture. Voluntary intake, increased in supplemented group, but had small effect on body weight change. Biuret in drinking water at 1% and 5% molasses was palatable. Water intake increased by about 25% on addition of molasses with biuret but without any effect on weight gain or fodder consumption. Supplementing urea @ 1% in molasses liquid drink to crossbred lambs grazing on cenchrus pasture resulted in better body weight gains than urea-salt-licks supplementation. Supplementation @ 30 g/kg  $W^{0.75}$ , of concentrate mixture and *ad libitum* intake of urea-molasses liquid drink, containing 26% crude protein resulted in average daily gain of 75 g. Commercial grade urea could be fed to growing lambs in the concentrate mixture containing barley and groundnut cake up to 2.6% of the concentrate mixture. The growth performance was about 84% of the control lambs. Enrichment of harvested cenchrus grass by ammoniation with 4 per cent urea improved the DCP value of the grass from 2.7 to 7.1 and TDN value from 47.3 to 55.8%. Feeding of treated cenchrus alone could maintain empty adult ewes. Hogget fed on chaffed and ground pearl millet stover alone or both the material ammoniated as such or ammoniated along with molasses indicated that the digestibilities of CP and CF improved significantly by ammoniation alone as well as ammoniation with molasses. The DCP value of the roughage increased from 4.5 to 11.0 and 3.6 to 13.0% by urea treatment alone and urea molasses treatment respectively. The treatment improved the DMI and energy value of roughage. Wool yield also increased from 1.1 to 1.4 kg by these treatments.



Ewes fed on untreated and 4% urea treated cenchrus grass along with 150 and 300 g babool pods improved in body weight gain. The animals receiving 4% urea treated cenchrus hay with 300 g babool pods showed better weight gain (14%) than their counterparts receiving only urea treated cenchrus hay which lost their body weights by 19%. The DCP and TDN content of cenchrus grass and ammoniated cenchrus grass was 1.6, 4.7 and 36.7, 39.0%, respectively. The respective DCP and TDN values with untreated and treated cenchrus grass along with 150 g babool pods were 2.6, 5.7 and 52.1, 56.4% and with 300g babool it was 4.1, 5.8 and 60.6, 58.6%. Treatment of bam grass (*Sorghum helepense*) with 4 per cent urea and subsequent stacking for 28 days improved the DCP and TDN values of the grass. Treated and untreated baru grass fed with or without 6 g  $Na_2SO_4$  per day indicated that sole bam feeding resulted in negative sulphur balance, whereas addition of  $Na_2SO_4$  maintained the animals in positive sulphur balance. Ewes maintained on varying levels of sulphur (0.09 to 0.23% of DM) indicated that addition of sulphur improved the digestibility of DM, CF and EE thereby increasing DCP



and TDN content. The sulphur balance was positive in animals fed 0.23% sulphur and negative in all others indicating that for optimum sulphur balance in sheep the diet should contain a minimum of 0.2% sulphur. Mustard straw has 2.5% crude protein and is not commonly utilized for animal feeding. Ammoniation and stacking (21 days) of mustard straw with 4% urea improved its CP content to 9.85%. In 90 days feeding with 200 g concentrate mixture resulted in loss of body weights in ewes. The DMI, DCP and TDN intakes ( $\text{g/kg W}^{0.75}$ ) were 62.2, 70.5; 2.6, 7.2; and 19.7, 34.0 in untreated and treated mustard straw fed ewes respectively. Wool yield (90 days clip) was 585.0 g in untreated mustard straw fed ewes 747 g and in treated mustard straw fed group. Weaner lambs fed on 50:50 concentrate and untreated and 4% urea treated mustard straw indicated that DM, DCP and TDN intakes in both the groups were far below their requirements for growth and in 100 days feeding period lambs fed untreated mustard straw based diet gained 3.0 kg live weight whereas the treated mustard straw based group had negative gain. Mustard straw fed with pearl millet, cenchrus grass and pala leaves along with 1% urea indicated that CP content of the feed combination ranged between 7.0 and 8.2%. Animals fed on 50:50 combination of mustard straw with pearl millet, cenchrus and pala leaves lost weights whereas all the four roughages in 25% incorporation was able to sustain the animals. DM, DCP and TDN intakes ( $\text{g/kg W}^{0.75}$ ) in these groups ranged from 41 to 57, 1.3 to 2.9 and 16.8 to 32.5, respectively. Urea treated mustard straw supplemented with either 200 g concentrate or 25% tree leaves can maintain the sheep.

Fungal (*Coprinus fimatarius*) treatment of ammoniated mustard straw improved its nutritive value and 5% urea with 75% moisture and 11 days incubation at 30°C was found to be ideal treatment for the straw. In vitro fungal culture techniques standardized and various white rot fungi used were *Ganoderma applanatu*, *Ganoderma lucidum*, *Longyites strata*, *Parva mentocela*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Pleurotus sajorcaju*, *Polyporus adustus*, *Polyporus arcularius*, *Polyporus sanguineus-154*, *Polyporus sanguineus-970*, *Polyporus versicolor*, *rametes hirsute*. Amongst these species *Pleurotus* and *Polyporus* sp. are the potential lignin degrading fungi, which can be exploited for the improvement of the feeding value of the mustard straw (low grade roughages) in sheep. Submerged system was set up using *P. sanguineus* and mustard straw (1%) in 0.02M sodium acetate buffered medium pH 4.5 with an incubation period of 29 days. *P. sanguineus* produced good amount of lignin degrading enzymes. The substrate having 70% moisture with supplemental 0.25% single super phosphate and incubated with white rot fungi at 35°C for 14 days improved IVDMD. Addition of urea found to have inhibitory effect on fungal growth. *C. versicolor* was found to be most suitable for biodelignification of mustard straw under solid-state fermentation and it improved CP content by 26% and DM digestibility (*in vitro*, *in sacco*) by 16%. Autoclaving of straw/roughages could be replaced by dipping straw in formaldehyde (500 ppm) and carbendazim (35 ppm) solutions for 8 hr for sterilization. To improve the nutritive value of mustard straw it was soaked in either water or boiling water or 2% NaOH solution for 6 or 12 hr. NaOH treatment reduced NDF, ADF and Cellulose significantly as compared to the water and boiled water soaking. The tenacity of structural fibre (g/tex) was found to be lower in NaOH treated mustard straw indicating structural changes brought about by NaOH treatment. When Mustard straw treated with different levels of urea (0, 3, 4, 5%), moisture (55, 65, 75%) and incubated at different levels of temperature (30, 40 and 500 °C) for 7, 14 and 21 days. The results indicated that there was a reduction in hemicellulose and lignin content of straw after treatment with urea, linearly.

When urea treatment was done using alternate layers of urea treated and untreated straws the ammonia produced was diffused to untreated layers, increasing concentration of nitrogen (% DM) in straw from 0.78 (untreated) to 1.74-2.06% (treated straw). The technique saved water and labour requirement. The hydrolyzation of urea to ammonia, during treatment in conventional method was 75-85%, which was similar in layer method whereas urea could not be hydrolyzed when concentrated urea solution was either poured or placed in the form of lump inside the straw. Spraying of urea solution on the sewan and drying it was found to be better method of feeding urea as compared to urea in drinking water. Urea: starch ratio 1.5: 13 was adequate and safe for maintenance of adult Marwari Sheep when sewan stover was fed as roughage. *In vitro* nutrient digestibility was lower in H<sub>2</sub>O<sub>2</sub> treated than NaOH treated



mustard straw (MS). The contents of OM, CP, ADF and cellulose slightly reduced and the per cent NDF, hemicellulose, lignin and *in vitro* digestibility marginally increased when the MS was soaked for different hours. On the other hand, non-significant differences were observed among periods of soaking for OM, CP, cell wall constituents and *in vitro* digestibility. The period and NaOH/ H<sub>2</sub>O<sub>2</sub> treatment did not have their combined effect on the nutritive value of MS. Alkaline H<sub>2</sub>O<sub>2</sub> treatment of mustard straw improved DM and digestible DM intake, decreased the N excretion in urine, higher N balance and these effects were more pronounced in 2% NaOH + 1.5% H<sub>2</sub>O<sub>2</sub> treated mustard straw. Exogenous enzymes fortification with concentrate did not significantly alter nutrient intake and utilization and the rumen fermentation attributes in rams fed Cenchrus hay based diets. However, pre-treatment of roughages at 30% moisture level showed promise through improvement in nutrient digestibility (OM, NDF, ADF and CP).

### **Nutrition-environment interaction**

Under heat stress the growing lambs, require additional energy to support the priority functions of thermoregulation and growth. The adult animals fed 115 and 130% above their maintenance requirement during summer under hot sun not only sustained their weight but could increase it at 130% of maintenance suggesting that the animals had enhanced energy requirement during summer. When subjected to graded nutritional stress Malpura and Chokla sheep and Sirohi goats, compared to Avikalin and Avivastra, with their lower body reserve lost more weight and took longer period to regain the lost body weight and the effect was pronounced in winter as compared to that in summer. The crossbred Mutton Synthetic lambs despite their higher energy expenditure in thermolysis, grew faster, utilized the feed efficiently and therefore had better productive performance. Two housing systems were evaluated, under shed and open corral with panipula shelter in 20% of the area. No apparent difference in feed intake and digestibility of nutrients, blood-biochemical profile, body weight changes and wool yield and quality was observed under the two housing systems.

### **Utilization of agro by-products in feed**

Water soaking of mustard cake reduced its glucosinolate, (6.53% in untreated) content to the extent of 12, 23, 29 and 36% at 1, 2, 4 and 6 hr, respectively, whereas 8 and 12 hr of soaking did not have added advantage. Lactating sheep fed on mustard cake incorporated concentrate feed found to contain 10-14.8 µg/ml of thiocyanate in milk and 5.2-6.5 µg/ml of thiocyanate in serum. Cotton seed cake could replace groundnut cake on equal nitrogen basis in lamb ration. Complete feeds have been developed containing de-oiled rice polish and urea for maintenance purpose; wheat bran has been used with pala leaves in 50:50, 75:25 for lambs which have gained 50 to 70 g/day. Sugar beat pulp at the rate of 53% and sea weed meals at the rate of 20% and wheat bhusa at the rate of 25% can replace expensive ingredients like maize, barley in the rations for lambs. Replacement of barley at the rate of 50 and 75% by molasses resulted in 235 and 157 g body weight gains. Guar korma may replace groundnut cake up to 35% in concentrate ration without any deleterious effect. Feeding of spineless prickly pear cactus along with roughages like cenchrus or grass can serve as sustenance ration for short term scarcity feeding.

### **Complete feeds for sheep and goats**

Complete feeds have been developed for sheep and goats incorporating roughages and concentrates in mash form or in pellets. Roughage components used in these feeds were ground cowpea hay, ground cenchrus hay, *Zizyphus nummularia* (Pala) leaves, *Ailanthus excelsa* (ardu) leaves and *Prosopis cineraria* (Khejri) leaves. Such complete feeds can be used for lambs and kids for meat production. Feeds containing 50% concentrates and 50% roughage have been found satisfactory, although high energy feeds can be prepared for higher growth with 60:40 or 75:25, concentrate to roughages ration. Feed conversion ratios of 5 to 8 with DM consumption of 3.5 to 5.0% of body weight have been obtained on feeding to lambs. The growth performance and feed utilization pattern of Malpura and

Malpura × Awassi, under complete ration and cafeteria system of feeding, indicated that the weaning weights of Malpura (11.25) and Malpura x Awassi (11.9 kg) were similar, but the finishing weight after 3 months of feeding were higher in Malpura x Awassi (25.4 kg) than Malpura (21.2 kg). The digestibilities of nutrients were higher in cafeteria system than the composite system of feeding. On a complete diet containing 40% siris pods, the digestibility of nutrients (DM, OM, CP, TCHO, NDF, ADF) and retention of nitrogen was significantly higher in goats than sheep. A complete diet was prepared with the ingredients, cenchrus straw (38.6%), dried ardu leaves (37%), wheat bran (14%), mustard cake (7.4%), common salt (1%) and mineral mixture (2%) and its performance was evaluated in Malpura rams and Kutchi bucks. The DMI (g/kg BW) was higher in sheep (21.48) than in goats (11.77). The goats, being browsing species had low DMI, when maintained under stall feeding. The digestibility of DM, NDF, ADF and GE was in goats than those of sheep. However, CP and hemicellulose digestibility followed a reverse trend. It is concluded that although cenchrus and ardu leaves based grain less complete diet had desired level of nutrients to sustain the animals but goats did not relish it. CFB were prepared using 80% FTL after mixing with other ingredients (mustard cake 6, wheat bran 6, uromol (with 16.7% urea) 6, common salt 1 and mineral mixture 1 parts) and pressing it at 4000 psi in complete feed block making machine. Animals failed to sustain body weight indicating further enrichment of the CFB. Fungal treated straw could be included in complete feed block and it did not produce any toxic symptoms in sheep indicating safe applicability and to harness improvement in fibre digestibility for higher nutrient uptake.



### Fodder conservation

When cowpea silage was prepared at the flowering and pod stage with or without molasses and fed to Malpura rams, palatability and dry matter digestibility of the silage was increased by the addition of molasses. Ensilage at flowering stage was better than at pod stage. Cowpea mixed with pearl millet crop (50:50) was harvested at pod stage and ensiled as chaffed untreated or treated with 5% molasses and 1% common salt by weight. Rams on an average consumed 2.52 and 2.34 kg dry matter/100 kg body weight from untreated and treated silages, respectively. There was no significant effect of molasses-salt treatment on dry matter intake and digestibility of nutrients except that of crude fibre which declined significantly. Silages from green neem leaves as such or with (super mindif; 2% dry basis) or with molasses (10%) and green oats with or without pala leaves can be fed to sheep. When green pearl millet fodder was ensiled with or without 0.50% biuret, Ammonia-N concentration increased fivefold. Although the crude protein content increased from 5.20 to 7.60, the palatability of the biuret-treated silage was decreased. The TDN value of the treated silage was also low.

### Processing of tannin rich feeds for their increased utilization

Fodder/feed obtained from trees commonly known as top feeds constitute an integral part of diets of sheep and goats in arid and semi-arid regions of India. Top feed simulate the good quality forages with respect to chemical composition, however, their utility has been found limiting due to large quantity of tannins. When leaves from *Acacia nilotica*, *Ailanthus excelsa*, *Zizyphus nummularia*, *Z. maritima*, *Calligonum polygonoides*, *Prosopis cineraria*, *Dichrostachys nutans*, *Ficus bengalensis*, *F. religiosa*, *Bauhinia racemosa* and *Leucaena leucocephala* were analyzed for their tannin content and chemical and biochemical nature of tannins, it was observed that except *A. excelsa*, all other leaves contained tannins. Although concentration of tannins varied with the season and age of the leaves, but pattern of change was not uniform with all the tree leaves studied. Chemical analysis of tannins showed the presence of tannins in the cell wall fraction (NDF and ADF) of tree leaves. Hence tannin content may lead to over estimation of lignin in tannin rich tree leaves. Tannin content and its protein precipitating capacity were mainly responsible for adverse nutritional effects. *P. cineraria* and *Z. nummularia* tannins had higher protein precipitating

capacity than *A. nilotica* and so the protein digestibility of the former was less than the latter. Tannins may combine with dietary and enzyme proteins and reduce digestion. Casein complex with tannic acid was less degraded by sheep rumen microbes. This inhibition of proteolysis of casein was alleviated with the addition of urea. Practical feeding experiments with *P. cineraria* + urea also showed that animals performed better in comparison to *P. cineraria* alone. Similar to tannic acid, *Z. nummularia* tannins also inhibited proteolysis of casein by rumen microbes. Looking at the role of tannins in nutrition, a simple method for the determination of tannins in intact tree leaves was developed for the use of feed industry. The method is based upon protein precipitating capacity of tree leaf tannins. Deleterious effects of tannins depend upon its ability to form complexes with protein and other macromolecules. Polyethylene glycol-4000 binds preferentially to tannins in comparison to proteins. Experiments with *Z. nummularia* and *P. cineraria* showed that treatment with PEG-4000 not only increased the feed intake and digestibility of nutrients but also the productivity of animals. In vitro studies also showed that the inhibitory effects of *P. cineraria* tannins on trypsin, chymotrypsin amylase, ruminal cellulase were recovered in the presence of PEG-4000. The beneficial effects of PEG-4000 were also visible in the status of iron, protein and albumin in the serum of sheep. Attempts to reduce the tannins content in tree leaves by NaOH and FeSO<sub>4</sub> treatments were found to be impractical. Effect of PEG-4000 treatment on intake and digestibility of nutrients, wool yield and body weight change is as follow:

Attributes	<i>P. cineraria</i> leaves		<i>Z. nummularia</i> leaves	
	Untreated	Treated	Untreated	Treated
Initial body weight (kg)	24.64	24.95	22.65	23.13
Final body weight (kg)	20.08	24.36	19.31	29.40
Dry matter intake(g/d)	750	1030	617	975
Dry matter digestibility (%)	29.00	32.36	41.93	46.35
Crude protein intake (g/d)	111	147	85	113
Wool yield (g/d)	4.94	6.53	0.37	0.62
Body weight change (kg)	-4.56	-0.59	-3.34	6.27

It is thought sometimes, that animals consuming tannin rich feeds continuously may develop defence mechanism in the form of synthesis and secretion of proline rich proteins in saliva. Though sheep on sole *P. cineraria* diet had proline rich proteins in their salivary gland but this defence was not adequate to cope up with the high concentration of tannins. High cost of PEG-4000 prohibits its application in routine feeding. A tannin resistant rumen microbe was isolated from the sheep rumen. Its growth was observed under strict anaerobic condition and in the presence of 0.5, 1.0, 2.0, and 3.0 mg tannins in 9 ml of RGC broth. Growth was slow and in presence of 3.0 mg and higher amount of tannins in the culture tube, bacteria did not grow. The degradation of tannins of *P. cineraria*, *B. racemosa*, *F. bengalensis*, *L. leucocephala*, *Z. nummularia* and *D. nutans* have been shown by the isolated bacteria.

### Feed toxins and food safety

Feed ingredients used at CSWRI Avikanagar for feeding of sector and experimental animals including the rations prepared from local market were tested for aflatoxin contents. The aflatoxin contents in all the ingredients are within the permissible limits of sheep feeding, however maize had highest aflatoxin content (337 ppb) compared to other ingredients. Mould infestation was least in DORB, adult pellets and lamb rations showing total aflatoxin content of 31.9, 29.0 and 29.0 ppb, respectively. Therefore, mycotoxicosis is not a problem for our livestock, interestingly pellet feed and lamb ration obtained from local manufactures were also safe for aflatoxin content. The damaged wheat also contained aflatoxin only 50 ppb seems greater possibility of feeding in sheep to economize cost of production.

Profile of different aflatoxin showed that aflatoxin B1 content ranged from 6.9 to 37.9 ppb and aflatoxin B2 from 10.8 to 120 ppb. Aflatoxin G1 was detected in til cake (45 ppb), GNC solvent (38.96 ppb), maize (217 ppb), wheat bran (9 ppb) and rabbit pellets (74 ppb), while aflatoxin G2 was detected only in til cake, GNC solvent and adult pellet feed.

Animal feeding trial on bio-safety studies with of Bt cotton crop using seed meal: Bt cotton is a transgenic plant, producing an insect controlling protein Cry1A(c), the gene which has been derived from the naturally occurring bacterium, *Bacillus thuringiensis*. Nutrient (OM, CP and fiber fractions) and mineral (Ca, P, Mn, Co and Zn) contents were identical in Bt-cotton and non-Bt cotton seeds. The growth performance of lambs was similar on control, non-Bt cotton seed and Bt-cotton seed included diets. The growing lambs consumed 168 g Bt-cotton seed per day and did not have apparent adverse effect on dry matter intake, nutrient utilization and nitrogen balance. Similarly Bt-cotton seed intake of 0.681% of body weight or 19.5% of dry matter intake did not produce deleterious effect on performance and dry matter intake, thus palatability and growth performance was not a problem for Bt-cotton seed feeding in lambs even under high plane of nutrition. Thus feeding of Bt cotton seed to lambs did not alter immunity and allergen status.

### Rabbit nutrition

For maintaining adult rabbit, a diet containing 13% CP and 2.20 Mcal/kg DE is recommended. A normal adult rabbit usually consumes 30-35 g DM/kg body weight. To avoid gastro-intestinal problems diet should contain 20-25% ADF and 12-15 CF%. For growing kits a diet containing 18-19% CP and 2.5-2.8 Mcal/kg DE is required to achieve a commercially viable growth rate of 28-30 g/d. The gestation length in a doe is one month and since the litter size at birth ranges from 7 to 10, a pregnant doe requires continuously high energy and protein in her diet. A 15% increase in DMI is generally observed during gestation over non pregnant stage.

Physiological stage	Requirement/kgW <sup>0.75</sup>			Nutrient concentration	
	DM (g/d)	DCP (g/d)	DE (kcal/d)	CP%	DE (kcal/kg)
Maintenance	42.6	3.80	94.7	13.0	2200
Growth*	91.0	13.0	25.5	19.0	2700
Gestation	45.0	5.9	105.5	18.0	2400
Lactation	64.0	9.0	170.0	18.0	2700

\*growth requirement of kit growing at 28g/d

A diet with 18% CP and 2.4 Mcal/kg DE was found sufficient to achieve desirable litter size. Litter weight accounted for about 12% of body weight of doe at kindling. A healthy kit grows about 8-10 g per day. To maintain this growth, lactating doe has to produce enough milk. The DM intake of lactating doe was about 35-50% higher in comparison to dry stage. For achieving desirable weight, a feed should contain leguminous green fodder and concentrate. A complete diet should have 18% CP and 2.7 Mcal/kg DE. Optimization of protein levels for various physiological stages in Angora rabbits: Adult British and Russian Angora rabbits (more than 6 month age) were fed for 9 months, 14.4, 16.2, 17.1, 18.1, 19.4, 22.0 and 23.1% proteins in their diets respectively. Wool yield was highest on 17.1% protein diet (0.60 g/day), followed by 14.4% protein diet (0.58 g/day). The digestibility of nutrients for crude protein increased with increasing protein content. There was no effect on fibre characteristics of the wool. Animals almost maintained their body weight throughout the experimental period. It was concluded that 17% CP is required in the diet of Angora rabbit for wool growth. Six week old male Angora rabbits were fed with basal diet of concentrate pellets at 60% of the total daily feed requirement and the rest 10% through green tree leaves of biul (*Grewia oppositifolia* Rorb). Kachnar (*Bahumia variegata*) and Winter/Kashmiri willow (*Salix* species) up to 12 weeks of age. No significant difference in weight gains, feed/DM consumption, efficiency of feed utilization and wool yield/kg body

weight was observed. The wool yield/kg body weight ranged between 14.79 and 17.60 g. Feeding rabbits on complete concentrate diets during pregnancy and lactation resulted in higher birth weights of kits and body weights subsequently at 6 and 12 weeks of age than those on concentrate + roughage (50:50) diets. The rabbits on completely roughage diets attained the lowest body weights. Both broiler and Angora breeds of rabbits on concentrate gained more than their counterpart on concentrate + roughage diet. Grey Giant gained more than Angora rabbits on concentrate diet. There was no significant difference in weight gains of Angora rabbits on concentrate + roughage. For grower meat rabbit protein contents of diet should be around 20%. Feed efficiency was also highest on 19.4% crude protein diet. Cowpea hay fed broiler rabbits expressed higher gain, consumed more feed and had higher feed efficiency of gain than those fed Kudzu vine and Robinia leaves. Dried tomato pomace can be incorporated in the diets of growing rabbits upto 20%.

When German angora (GA) rabbit weaners were fed supplement diets with 0, 25, 50, 75 and 100 gig of Kenzyme - a mixture of p-glucanase, oc-amylase, lipase, cellulase, protease significant  $P < 0.05$ ) improvement of 47.06, 36.54 and 14.62% in 84 days weight, feed conversion ratio and wool yield (3-shearing) were observed in treatment group supplemented with 75 gig of Kenzyme. The average daily gain (ADG) also significantly ( $P < 0.05$ ) regressed over Kenzyme intake in the same group. The digestibility of organic nutrients also improved showing that Kenzyme level of 75 gig was beneficial as supplement in feeds of Angora rabbits. Rice-phak at 20% level along with barley 11% do not form a successful replacement of maize + wheat bran + rice polish in the feeding regime of Angora rabbits. Methionine supplementation resulted in higher gain, better feed conversion and dressing percent in animals receiving the test diet. Adult Soviet Chinchilla rabbits could be maintained on paper mulberry leaf based ration having 18% crushed barley. Robinia leaves could be added @ 25% for improving gains in Soviet Chinchilla broiler rabbits. Kudzu vine may be included up to 25% level in adult White Giant broiler rabbit ration without impunity. For Soviet Chinchilla kits weaned at 28th day of age 4% fat as palmolene oil for growth is recommended in the feed. Ground leguminous fodder (20-25%) along with a mixture of cake, bran, molasses, salt and mineral mixture can be used as pellet complete diet. Cowpea hay, lucerne hay and berseem hay can be used interchangeably in the complete diet. Bam, khejri, pala, mango, pipal, bargad and ardu, tree leaves can be used with 15% in complete diet for satisfactory performance of rabbits. Maximum gain can be obtained on mulberry based concentrate feed. Best average daily gains were achieved when green leguminous fodder was fed ad lib along with concentrate pellets. The feed pellets contained 20-22% CP and 3.0 Mcal/kg DE. The carrots were also found suitable as a source of green fodder. Dry roughage (cowpea, cenchrus) or tree leaves (mulberry, khejri, ardu) were fed along with concentrate to growing rabbits. The intake of dry fodder hay was least (18 g/day), with cenchrus and maximum (38 g/day) with mulberry leaves. However, all animals could achieve similar finishing weight at 12 weeks of age. The rabbits on cenchrus diet compensated the nutrient requirement by higher intake of concentrate. The feasibility of replacing pellet feed with soaked soybean and maize without and with heat treatment in broiler rabbit was studied. No ill effect of feeding soaked and soaked and boiled soybean feeding was observed on rabbit's health.



## Physiology and Biochemistry

**S.M.K. Naqvi and Davendra Kumar**

The work on ovine physiology and reproduction was initiated two years after the inception of the institute but the impetus on research was given after the establishment of the Physiology Section in 1965. At the onset the main thrust of research was on environmental physiology and applied reproduction which was further enlarged in 1975 by up gradation of the section to the status of the division. The division was subsequently renamed in February 2003 as the Division of Physiology and Biochemistry after merger of Biochemistry Section with the division.

The major programmes undertaken were on ram semen preservation, artificial insemination and adjudging the physiological adaptability of different strains developed at the institute. During the IX and X plan periods, the main emphasis was geared up on research on developing advanced reproductive technologies pertaining to cryopreservation of ram semen, embryo technology and control breeding in ewes while the work on adaptation of sheep and rabbits to hot semi-arid environment. The division is credited to report for the first time in the country the birth of lamb following transfer of frozen-thawed embryo in 1998 and IVM-IVF-IVC produced embryo in 1999. The division has also been successful in producing 240 elite lambs through ETT and cryo-conserving 263 *in vivo* produced embryos from various sheep breeds.

The division has successfully developed number of protocols suitable to Indian breeds of sheep. The notable research contribution was on development of techniques pertaining to cost-effective indigenous progesterone impregnated intra-vaginal sponges, laparoscope aided embryo transfer and intrauterine artificial insemination, superovulation, ram semen freezing and transcervical artificial insemination. The two technologies namely, '*Artificial insemination of sheep using short-term preserved ram semen*' and '*Estrus induction and synchronization in sheep*' have been successfully demonstrated and transferred in the field. The kit developed for '*Estrus synchronization in sheep and goats*' has also been commercialized. The scientists of this division have filed two patents through ICAR and conferred with prestigious awards from ICAR, ISCA, NAAS and other scientific societies in national and international seminars. The two research contributions of the division have been published as *records* in the *Limca Book of Records*.

Various physiological studies have been conducted on different aspects of reproduction and adaptation of sheep and rabbits during last five decade. The important findings of these studies are as follows:

### **Semen production**

Rams of native breeds start donating semen at 6-7 month of age. Hot weather has deteriorating influence on semen quality of exotic rams. It could be overcome by acclimatizing the rams for three generations. Exotic rams donate good quality semen at the rate of one ejaculate per day up to 19 days. Second collection of the ejaculate within 10 minutes interval increases the volume of semen and number of spermatozoa. Semen of exotic rams at 2-3 years of age is best and limit is up to 5 years. Foot and Mouth Disease, Sheep Pox and Blue Tongue even at sub clinical level affect the semen quality. Stresses like dipping, vaccination etc., also affect the semen quality adversely. Saxome (Indian herbs) at 7g/day along with 150 g concentrate for 40 days improve the *libido* of the rams through its aphrodisiac, general stimulant and spermatogenic action. The influence of *FecB* gene introgression on sperm production and quality was reported for the first time. First time reported the influence of age on CASA derived sperm motion characteristics of ram lambs raised under intensive management system in a semi-arid tropical environment.

### Biochemistry of semen

Calcium, Sodium, Potassium, Inorganic Phosphorus, Chloride and Alkaline phosphatase activity in semen do not vary between breeds. Electrolytes in the semen did not affect the physical attributes. Creatinine value of semen is 4 times higher than the serum. Glutathione level in blood of Malpura animal is double than Nali and Chokla. Acid phosphates activity in semen is inhibited by the excess of phosphate ions and RNase by the acetate ions. Amalyse activity depends on breed and season with highest activity during spring followed by autumn.

### Reproductive efficiency

The twinning rate achieved in Patanwadi sheep were 30.7 and 25.0% in the ewes mated in natural and induced estrus, respectively. After first lambing, second breeding was successfully performed in 58.3% ewes with in targeted eight months period ( $232.7 \pm 2.14$  days). All the ewes were mated second time within inter-service period of  $271 \pm 9.68$  days. Estrus response could be achieved more than 90% in Malpura ewes during breeding season by using different estrus synchronization protocols (PGF<sub>2</sub>α, P<sub>4</sub> sponge + 200 IU PMSG and P<sub>4</sub> sponge only). Feed supplementation @1.5% of body weights affected the estrus and ovulation responses in the ewes. Feed supplementation during summer season improved ovulation rate ( $1.2 \pm 0.20$ ) compared to control group ( $0.7 \pm 0.15$ ). Intensive feeding of lambs increased the testicular development to donate semen at 7 months of age and attain the puberty at 9 months of age in females.

### Lactation studies

Malpura ewes produce maximum milk (65 kg) in 90 days of lactation whereas Chokla yielded 24% less than Malpura. Milk yield of Rambouillet and crossbred is similar to Malpura. Peak milk yield is achieved during 2<sup>nd</sup> week of lactation.

### Physiology of growth

Malpura lambs require 6.5 kg of milk to produce one kg live weight. Crossbreds are more efficient in milk utilization. Weaning of lambs at 45 days or 75 -90 days result in similar body weights at 6 and 9 months of age. Administration of thyroxin improves body weight and fertility in ewes. Castration at birth does not improve weight gain up to 6 months and dressing yield. Castration at 3 or 9 months do not affect carcass yield. Body condition score (BCS) of 3.0-3.5 of ewes is optimum at mating and during gestation for achieving higher lambing and birth weight of lambs. Rams kept under the conditions of a hot, semi-arid environment should be maintained at moderate (3.03.5) BCS during breeding stage to ensure maximum reproductive efficiency.

### Effect of water deprivation

Offering water at intervals of 48 hr for 6 months and 72 hr interval for a prolonged time affected lamb growth, pregnancy, lactation and carcass yield. However, it did not affect non pregnant, non lactating and non growing animals. There is no body weight loss in Chokla sheep when watered at weekly intervals. During winter watering at intervals of 48 hr improved digestibility of DM, CP, EE and CF. The animals avoid intake of high protein materials during restricted watering. Watering at intervals of 72 hr reduces intake of DM and consequently body weight. Restricted watering, increases concentration of salt in urine.

### Adaptation physiology

Chokla and Chokla synthetic sheep responded similarly and fairly tolerate the thermal stress (40°C for 6 hr/day). Animals under energy crisis experienced greater degree of stress. *Ad libitum* feeding and exercise increases



greasy fleece production. Avikalin sheep can tolerate ambient heat stress during summer months provided there is no nutritional stress. Solar exposure to animals results in minimal loss of body growth performance and animal shows ability to compensate after removing the stress. Thermal and nutritional stress had an adverse effect on physiological responses, blood parameters and reproductive efficiency of ewes. Thermal stress has greater effect on depleted sheep than *ad libitum* fed sheep. Nutritional stress has more effect than thermal stress and when both these stresses are coupled, it has more severe effect. When nutrition is not a limiting factor, then ewes are able to better cope up with thermal stress.

### Rabbits

Grey Giant and Soviet Chinchilla rabbits when bred on day 0 after kindling perform better with highest number of kits born and weaned as compared to rabbits bred on the 14<sup>th</sup>, 28<sup>th</sup> and 42<sup>nd</sup> day after kindling. German Angora rabbits produce poor quality semen during summer months adversely affecting the reproduction efficiency. Bucks sheared at one and 3 months interval and injected 0.2 ml GnRH every 48 hr for 6 times perform better.

### Technologies developed

The important technologies emerged out of the research conducted are as follows:

#### *Estrus synchronization in ewes by prostaglandin F<sub>2</sub>α*

A protocol developed for synchronization of estrus in cycling native ewes includes administration of two intramuscular injections of PGF<sub>2</sub>α @ 7.5 mg / kg body weight at 10 days interval (Fig. 1).

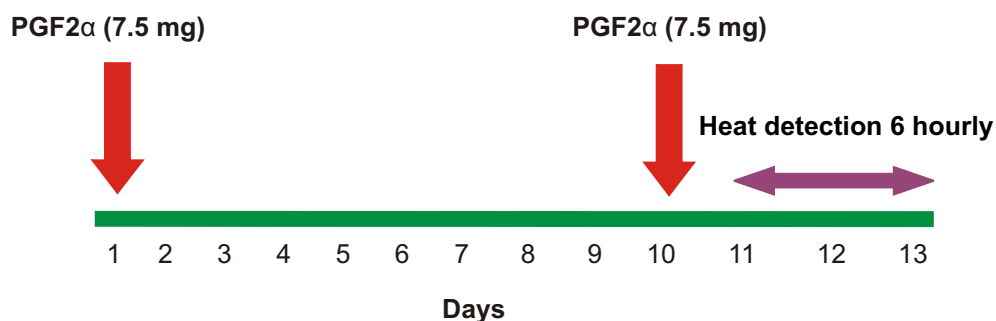


Fig. 1. Estrus synchronization protocol using PGF<sub>2</sub>α

#### *Intra-vaginal sponges for estrus induction and synchronization*

Progesterone impregnated intravaginal sponges (synthetic sponge foam and cotton thread) have been developed indigenously for estrus induction and synchronization in sheep and goats. Flat sponge was cut into circular pieces of similar shape in two sizes; Size 'A': 35 mm diameter and 27 mm height, Size 'B': 42 mm diameter and 27 mm height and were tied with 16-20 cm cotton thread. These sponges were sterilized by autoclaving. A solution of progesterone (0.175 g/ml) was prepared in ethanol and each sponge was loaded with 2 ml solution. The sponges were dried in hot air and kept in a sealed polythene bag until use. At present these sponges are prepared (Fig. 2, 4) and being used in routine in our estrus induction and synchronization programmes (Fig. 3).



Fig. 2. Estrus synchronization kit

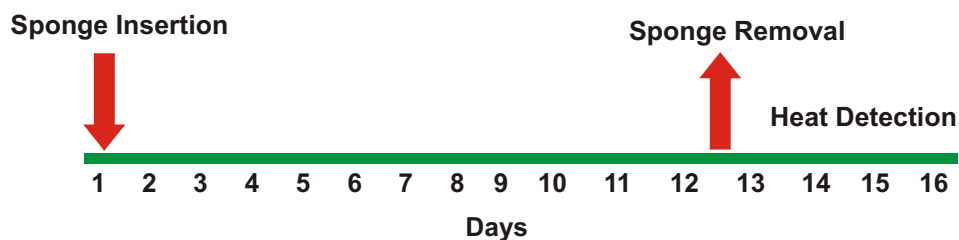


Fig. 3: Estrus synchronization protocol using intravaginal sponges

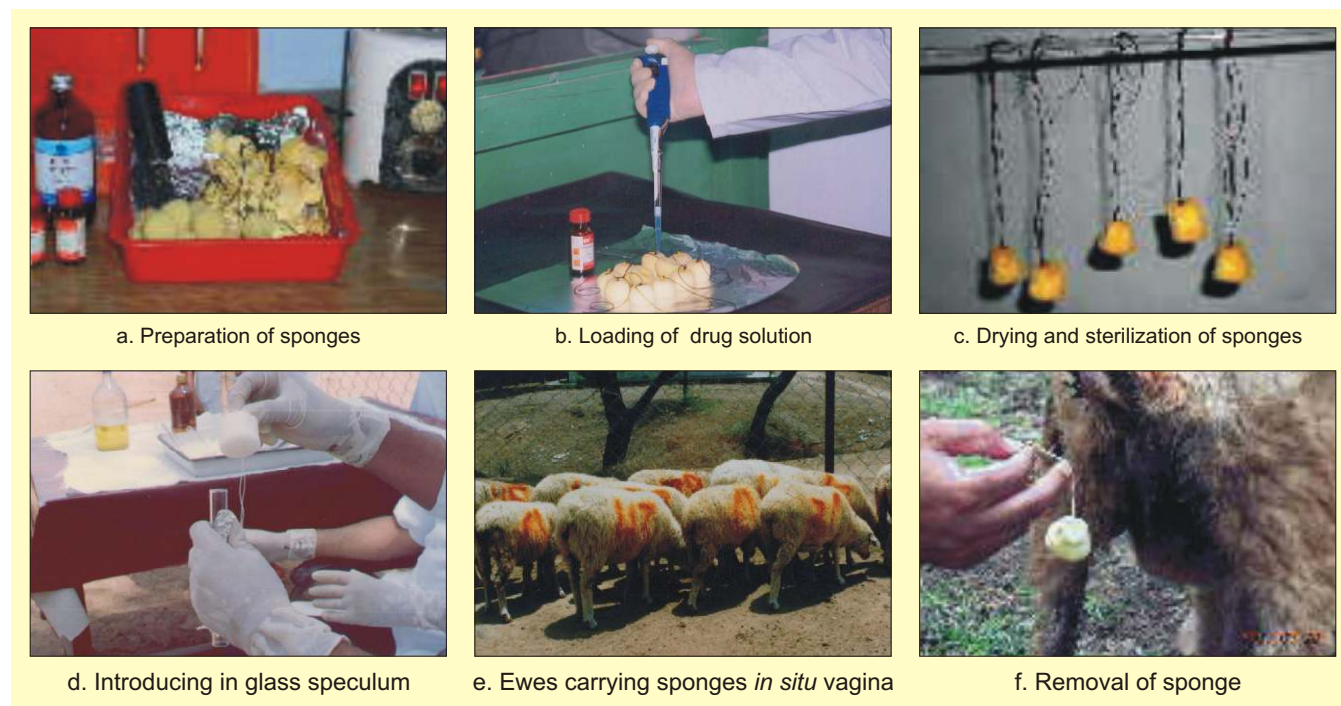


Fig. 4. Development and application of intravaginal sponges

The synchronization kit has been commercialised since 2006. Every year indigenous vaginal sponges are being prepared and supplied for use in TOT area of our Institute and in organised sheep and goat farms. More than 12000 sponges have been supplied to many research institutes, KVK's, Veterinary colleges, state government farms and to others for use in research and development work. These sponges had been utilized for fertility management and artificial insemination (AI) programme. A standard protocol has been developed for estrus synchronization of crossbred and native sheep breeds through progesterone impregnated vaginal sponges.

#### Superovulation protocol for native and crossbred sheep

To reduce the cost of superovulation and to minimize individual variability in response, a unique protocol consisting of FSH and PMSG combination was developed, standardized and validated for inducing superovulation in native and crossbred sheep (Fig. 5). Ovagen (5.4 mg) in 8 doses over 4 days (12 hr apart) in conjugation with PMSG (200 IU) resulted in acceptable superovulatory response (>8- 10 CL/ewe) in donor ewes (Fig. 6).

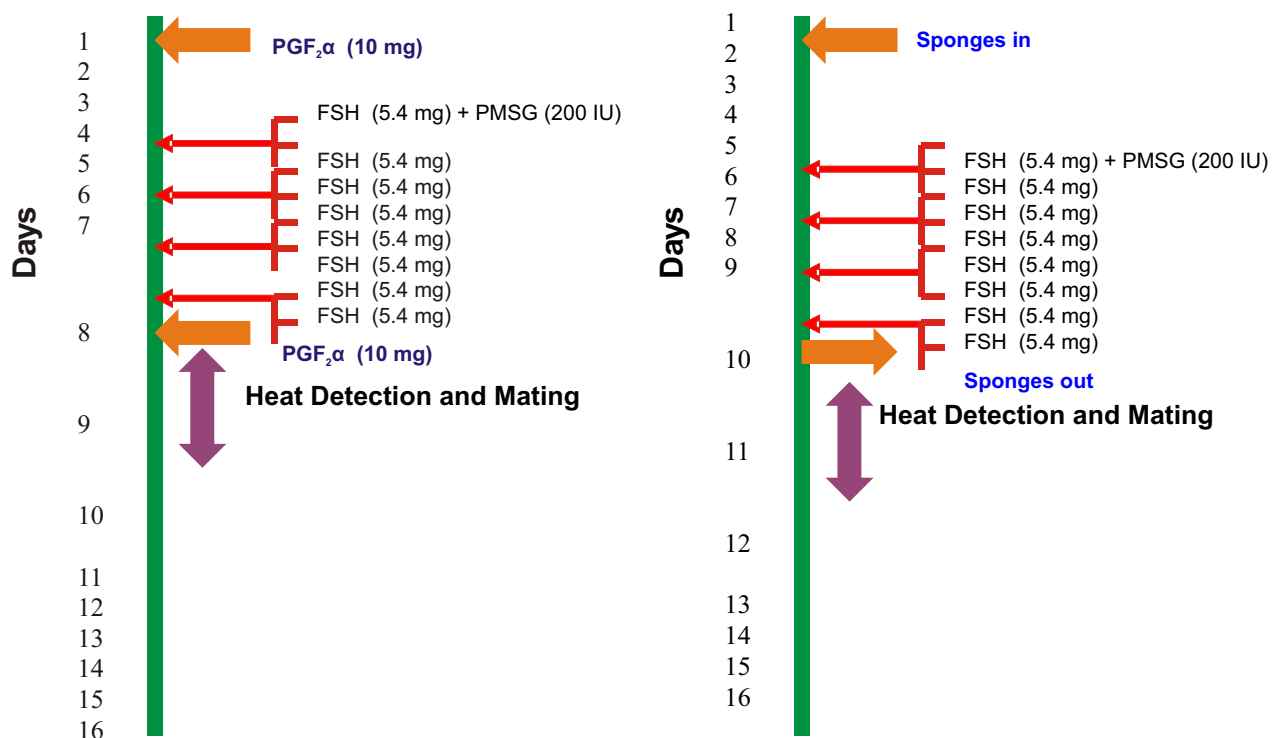


Fig. 5. Superovulation protocol in ewes



Fig. 6. View of corpus luteum (CL) on the sheep ovary

### Laparoscope aided embryo transfer in sheep

A quick and easy method of laparoscope aided embryo transfer was developed at the Institute. A special designed cradle was fabricated for easy restraining and positioning of ewe (suspended at 45° angle) subjected to laparoscopy. The recipients having synchrony with donor are fasted for 24-36 hrs before laparoscopy. Sedation is induced with intramuscular injection of xylazine. Xylocaine is administered subcutaneously as local anesthetic. Abdominal area anterior to udder is shaved and sprayed with 70% alcohol. Uterine horn ipsilateral to corpus luteum is gently grasped with the forceps (modified in our laboratory) and lifted through the puncture site to expose a loop of horn. A blunt needle is used to make a small aperture in the loop of horn and tom-cat catheter loaded with embryo is introduced into lumen of horn. Embryo is pushed into the lumen and horn is again put into abdomen. Skin is sutured or

sealed with wound clippers. This technique does not require costly equipment for inflating abdomen for laparoscopy and special imported atraumatic forceps and other equipments (Fig. 7-10). A total of 222 lambs belonging to Bharat Merino, Malpura, Kheri, Chokla, Munjal x Malpura crosses and Garole breeds were produced through *in vivo* derived embryos.



Fig. 7. Location of uterine horns through laparoscope



Fig. 8. *In vivo* produced transferable sheep embryos



Fig. 9. Transfer of embryos by tom-cat catheter into lumen of horn



Fig. 10. A- Large size Awassi x Malpura recipient ewe and small size Garole donor ewes; B- Garole lambs produced after *in vivo* derived embryo transfer

### Laparoscope aided intrauterine artificial insemination

A simple method of laparoscope aided intrauterine artificial insemination using frozen semen was developed in sheep. This procedure requires approximately 3-5 minutes for single insemination. Lambing rate of 35-44% is achieved following intrauterine deposition of frozen -thawed semen in Malpura ewes.

### Artificial insemination in sheep with liquid chilled semen

The techniques for fixed time AI in sheep have been developed, simplified and improved during the last few years (Fig. 11). Nevertheless, AI has not yet received universal acceptance in sheep industry due to several technical and economical reasons. However, with the advent of recent advances in reproductive technologies, the efficiency of AI in terms of conception and lambing rate has improved. AI has direct impact on the production level of the resultant progeny and improves the desirable production traits like body weight, wool quality, wool quantity and fecundity of sheep.

*AI with liquid semen:* To date the success of AI in sheep is limited to liquid semen. The main methods of short-term preservation of ram semen in a liquid state are based on storage at reduced temperature (0-5 or 10-15°C) by

reversible inactivation of spermatozoa. The lambing rate following use of liquid semen stored for one, two and three days are 45-50%, 25-30% and 15-20%, respectively.



Semen collection by artificial vagina



Evaluation of semen



AI of sheep with liquid semen

**Composition of semen diluents**

**Egg yolk- tris-fructose diluent**

Tris (hydroxy methyl) amino methane	3.634 g
Fructose	0.50 g
Citric acid monohydrate	1.99 g
Strepto-penicillin	0.3 g
Egg yolk	14 ml
Double glass-distilled water to	100 ml

**Egg yolk-glucose-citrate diluent**

Sodium citrate dihydrate	2.37 g
Glucose	0.80 g
Strepto-penicillin	0.3 g
Egg yolk	20 ml
Double glass-distilled water to	100 ml

**Egg yolk McIlvaine glucose diluent**

Disodium hydrogen orthophosphate	1.78 g
Citric acid monohydrate	1.68 g

Dissolve both separately in glass-distilled water to 100 ml. Take disodium hydrogen orthophosphate solution in 250 ml beaker and record pH. Add citric acid solution drop by drop and mix thoroughly. This will cause drop in pH. Continue adding citric acid till the pH becomes 7.0. This will form McIlvaine buffer

McIlvaine Buffer	100 ml
Glucose	0.8 g
Strepto-penicillin	0.3 g
Egg yolk	20 ml

Fig. 11. Steps involved in artificial insemination of sheep

**AI at farmer's flock:** Institute launched an AI programme for improving rural sheep of semi-arid region using liquid semen of exotic Awassi and native Patanwadi and Malpura rams. Semen was obtained daily in the morning from adult rams. Ejaculates having good semen quality were diluted 1:1 with EYMG diluent and stored in ice containing semen shipper. AI was done daily in estrus ewes for one cycle by depositing 0.1 ml containing 100 million sperm per os. AI with Awassi ram semen was performed in ewes exhibiting natural estrus while with Patanwadi ram semen in ewes exhibiting estrus after induction and synchronization. A total of 531 ewes from farmer's flock were inseminated with overall lambing of 60.6%.



*AI at organized farms:* AI with fresh diluted semen has also been conducted by the institute at organized sheep farms. At CSWRI a total of 466 inseminations were done in Malpura ewes at natural estrus with Garole semen between the year 1997 and 2006, which resulted in 52.4% lambing. At Asad Farm (Anantpur district, Andhra Pradesh), a fertility trial was conducted during autumn 2007 using fresh diluted semen of Dorper rams. Semen samples of good quality obtained from Dorper rams were evaluated and diluted 1:1 with Egg yolk citrate glucose (EYCG) extender. Fixed time AI was performed in 586 ewes of Nellore Zodpi breed after synchronization of estrus using 0.1 ml (100 million sperm) of fresh diluted semen, which resulted in 67.2% lambing.

### Validation of computer-aided semen analysis technique for assessment of motion characteristics of ram spermatozoa

The computer-aided semen analysis (CASA) technique was standardized and validated for objective assessment of sperm motion characteristics of ram spermatozoa. The precise results obtained by this technique were useful to evaluate sperm motion characteristics, which are not apparent by routine subjective evaluation of semen but are useful in assessing the fertilizing ability of ram sperm for its use in AI. The technique has been used for assessment of sperm motion characteristics and track dimensions of growing ram lambs; *FecB* carrier prolific rams and rams of different native, exotic and crossbred breeds during breeding and non-breeding season. The technique has also been applied to determine the influence of short-term preservation, freezing and thawing on motion characteristics of ram spermatozoa.

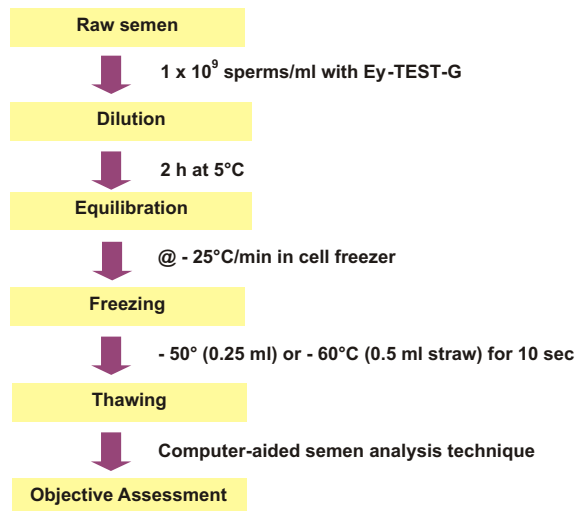


### Development of protocol for ram semen freezing using programmable cell freezer

A common protocol has been developed for cryopreservation of ram semen of exotic, crossbred or native breed packaged in medium (0.5 ml) and mini (0.25 ml) size French plastic straws. The variables optimised in the laboratory for developing the protocol are summarized below:

Variable	Optimum Conditions
Extender	Egg yolk tes tris glycerol (Ey-TEST-G)
Extension rate	@ $1 \times 10^9$ sperms/ml
Packaging size	0.25 ml (mini) and 0.5 ml (medium) straws
Equilibration time	2 hr
Mode of freezing	Programmable LN <sub>2</sub> vapour freezing
Equipment	Cell Freezer
Thawing temperature	50°C for 0.25 ml straw, 60°C for 0.50 ml straw
Thawing time	10 seconds
Assessment	Objective by CASA technique
Mean post-thaw motility	70 %

The schematic flow diagram of various steps involved in cryoprocessing of ram semen by this protocol is as follows:



The freezing protocol is based on controlled cooling and freezing, directly in the programmable cell freezer and CASA techniques. The steps involved are as follows:

Start temperature : +25°C

- Step 1 : Hold for 10 minutes (rate is zero and the temperature is maintained at +25°C), no seeding selected
- Step 2 : -0.15°C/min to +5°C
- Step 3 : Hold for 10 minutes (rate is zero and the temperature is maintained at +5°C)
- Step 4 : -25°C/min to -125°C
- Step 5 : Hold for 10 minutes (rate is zero and the temperature is maintained at -125°C)

The application of this protocol in all the breeds tested at the Institute gave 70 % average mean post-thaw motility of spermatozoa. The high post-thaw recovery is attributed to the criteria of processing only those ejaculates for cryopreservation which have thick consistency, rapid wave motion, i.e. having 90% initial motility and  $\geq 3000 \times 10^6$  spermatozoa per ml.

### Transcervical artificial insemination (TCAI) in sheep using frozen semen

Insemination cradle is a pre-requisite for TCAI technique. At the outset, a simple cradle was designed and fabricated locally to ideally suit TCAI of native sheep breeds with frozen semen. The simple and cost-effective design of this cradle makes it ideal and economic for large scale production to meet future demands and can also serve as a valuable import substitute. In this cradle, ewes exhibiting oestrus are restrained with hind quarter elevated so that the vulva is positioned at an angle of 80 to 90° i.e., vertically. Other insemination devices suitable to native sheep, such as speculum with plunger, catheter with bent tipped needle and miniature light source for TCAI were also fabricated in our laboratory (Fig. 12-16). To initiate the insemination the speculum with plunger is lubricated with a small amount of

medical gel and introduced into the vagina. Following TCAI with frozen ram semen progressive success has been achieved for the first time in this country in terms of lambs born and the results obtained in our laboratory are at par with the leading research teams of the World. Out of 293 sheep inseminated with frozen semen through TCAI technique, a total of 51 lambs (17.41% lambing) were produced.

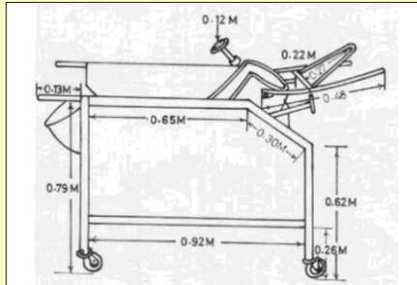


Fig. 12. A prototype of cradle designed and fabricated for TCAI



Fig. 13. Sketch of the operator performing TCAI in sheep



Fig. 14. Equipments fabricated for TCAI



Fig. 15. Location of os-cervix with the aid of light and introduction of needle for deep deposition of semen into uterus



Fig. 16. Silicone mould of vaginal canal

### Cryopreservation of ovine embryos and birth of lambs

*In vivo* derived embryos from native and crossbred sheep were cryopreserved using standard protocol. Embryos at the stage of morulae and beyond with good and fair morphology were selected for cryopreservation. Total number of sheep embryos (Bharat Merino, Malpura, Kheri, Chokla and Garole breeds) frozen and cryopreserved at CSWRI were 263 (220 by slow freezing and 43 by vitrification). In a major breakthrough our team of scientist from CSWRI was successful in producing a lamb from cryopreserved embryo for the first time in the country. Detail of lambs produced from frozen thawed embryos is as under:

Breed (Donor)	Days of embryo storage	Breed (Recipient)	Lamb	
			Sex	Birth weight (kg)
Bharat Merino	260	Kheri	M	3.9
Malpura	565	Malpura	M	3.8
Bharat Merino	191	Malpura	M	3.8
Malpura	180	Malpura	F	2.4



### Design and fabrication of sponge and applicator for large ruminants

Silent estrus and anoestrus are the major problems associated with large ruminants especially with buffaloes in TOT areas of the institute. For induction and synchronization of estrus in buffaloes a low cost but efficient device is required. Intravaginal sponge impregnated with progestogen and applicator (vaginal speculum and plunger) suitable for buffaloes in field were designed and fabricated. The applicator and sponges were made available to TOT workers for use in buffaloes.

### Major scientific breakthroughs

- ❖ A freezing protocol based on total control of cooling and freezing rates was developed for *ex-situ* conservation of sheep breed in the form of spermatozoa.
- ❖ Non-invasive transcervical artificial insemination technique was developed and refined for utilizing frozen ram semen.
- ❖ Low cost indigenously developed intra-vaginal progesterone impregnated sponges were developed for estrus induction and synchronization in different livestock species.
- ❖ Use of Gonadotrophin (FSH, PMSG) and Gonadotrophin releasing hormone (GnRH) in various combinations was successful in inducing multiple ovulations in native sheep.
- ❖ Multiple ovulations were observed in ewes immunized with 30 mer inhibin peptide and its effect persisted profoundly for 3 years as exhibited in terms of 5.3 ovulation rate.
- ❖ Recovery of 24 *in vivo* produced Garole embryos of transferable quality in a single flush was reported. This is the highest recovery for any embryo donor sheep breed reared under tropical environment.
- ❖ Garole lambs born of significantly higher birth weight were reported on transfer of Garole embryos in to large size non-prolific recipient ewes.
- ❖ Birth of first lamb in the country was achieved from frozen sheep embryo.
- ❖ Full-term lamb was produced for the first time in the country from embryo derived from IVM-IVF-IVC techniques.
- ❖ Awassi and Patanwadi crossbred lambs were produced in farmers' flocks through artificial insemination technique using liquid semen.

### Patents

Title	Details of Patent/Registration/ Commercialization	Year
A simple cradle for transcervical artificial insemination in sheep	A patent application submitted to the patent authority on behalf of the council has been accepted as an IPR of ICAR (203/Del/2000). Name of Inventors: S.M.K. Naqvi, A. Joshi, J.P. Mittal	2000
Indigenous production of progesterone impregnated vaginal sponges for sheep	A patent application submitted to the patent office, Govt. of India vide F.No. 1(36)/2004-IPR dated 7 <sup>th</sup> Oct., 2004 through IPR section, ICAR (1833/Del/2004). Name of Inventors: S.M.K. Naqvi, R. Gulyani, J.P. Mittal, V.K. Singh	2004
Low Cost, Indigenous Cradle for Safe Restraining of sheep	Patent applied on 16-11-2009 in ITMU, CSWRI, Avikanagar (2113/Del/2004). Name of Inventors: V.P. Maurya, V. Sejian, S.M.K. Naqvi, S.A. Karim	2010
Low Cost, Indigenous vaginal sponges for estrus control in buffaloes	Patent applied on 09-08-2010 in ITMU, CSWRI, Avikanagar (2114/DEL/2010)] and published in IPR Gazette (Publication date-07.10.2011) Name of Inventors: S.M.K. Naqvi, S. Singh, D. Kumar	2010



## Animal Health

**B.N. Tripathi and C.P. Swarnkar**

The Division of Animal Health has its origin from the Sheep Husbandry Section initiated since inception of the Institute in 1962. In 1970, the Sheep Husbandry Section was renamed as Sheep Health Section. Up to 1978, the main role of section was to provide health cover to the flocks of the Institute. The section initiated a long-term research project on the epidemiology of sheep and goat diseases in 1978. The section has provided valuable insight on the various diseases and health related problems that occur when the production potential and genotypes of animals are progressively altered. With the passage of time, the diagnostic laboratories were also developed to fit into the requirement of the Institute. The pathological and parasitological diagnostic work gained precedence to provide initial. Later, the bacteriological and virological diagnostic work was initiated. The Animal Health Section was re-designated as Division of Animal Health in 1987. The division initiated two long term projects: (i) "Application of the appropriate flock health technologies for sheep and rabbit" and (ii) "Diagnosis and investigation of sheep and rabbit diseases", since 1992. Development of rapid diagnostic facilities for epidemiological studies, disease surveillance and monitoring, forecasting, prevention and control of sheep and rabbit diseases in the Institute / field flocks was undertaken.

### Microbiology

The bacteriological laboratory is equipped to conduct routine bacterial isolation and identification. Antibiotic sensitivity test is routinely employed in infections with suspected bacterial species. This testing benefited in scheduling, to a certain extent, the antibiotic sensitivity profile and prevents indiscriminate use of common antibiotics. During last five decades, general resistance to common antibiotics have appeared in preventing pathogenic organisms in the Institute sheep population, whereas the antibiotic sensitivity trend has been quite different in isolates from field flocks. Trend observed has been of antibiotic resistance against strains of *E. coli*, *Pasteurella* and *Corynebacterium* sp. to common antibiotics like penicillin, streptomycin and tetracycline. However, they are variably sensitive to ciprofloxacin and chloramphenicol.

*Pneumonia and pneumoenteritis complex*: Acute pulmonary disease has been a serious problem in sheep flocks since inception of the Institute. Usually mortality due to respiratory syndrome starts in March and reaches at peak in May. Losses were at the maximum in suckling (0-3 months) mostly with non-suppurative lesions. However, incidence of pulmonary suppuration and formation of abscesses increases with the age.

In general the agents implicated in etiology of respiratory syndrome include a wide spectra of viruses such as PI 3, Adeno and Reo viruses, bacterial species such as *Hemophilus*, *Bordetella*, *Pasteurella*, *Corynebacterium*, *Mycoplasma* and *Chlamydia* along with a chain of predisposing factors mainly the environmental and managerial fluctuations. In this duration presence of only few of these agents could be ascertained in the Institute flocks. Screening of sera of the flocks against Ovine adenoviruses (OAV) antigen revealed 8.62% prevalence rate in sheep. Higher prevalence in exotic animals followed by higher crosses and then the half bred was also found. Incidence of reactors to OAV antigen was highest in the suckling followed by weaners and hoggets. However, no relationship could be established between clinical status of the animals and positivity of the reaction to OAV, but role of OAV as a primary infectious agent for pneumoenteritis complex has been proved in studies carried out in various parts of the world. The initiation by the viral agent is further complicated by secondary infections like *Pasteurella*, *Pseudomonas* and *Corynebacterium*. Involvement of PI virus, the most common virus implicated in the complex etiology of pneumonia, however, could not be ascertained at Avikanagar but its role in sheep and goat pneumonia has already been proved in the country.

Pasteurellosis is a well known cause of pneumonia and septicaemia in sheep with high morbidity and mortality. From CSWRI, Avikanagar, *P. multocida* serotype B was isolated from an outbreak of pneumonia and it was compared with a reference strain obtained from FAO regional reference laboratory, Sri Lanka. Protein profile of capsular antigen of the isolate extracted by different methods was studied. Indirect ELISA was also standardized by using the capsular antigen which can be used in sero-surveillance of pasteurellosis in the flock. Similarly from a field outbreak, *P. haemolytica* serotype A was isolated and studies on the protein profile of the capsular antigen was carried out.

Mycoplasma strains have also been isolated from sheep having respiratory distress and typed as *M. agalactiae* by using reference sera (National Collection of Type Culture, London). The isolates were found to be pathogenic in experimental trials that indicate their primary / secondary involvement in causing pulmonary diseases. Studies on serum antitrypsin activity in pneumonic sheep revealed that double fold increase in the level of total trypsin inhibitor with clinical signs are better parameter for detecting the acute pneumonia in sheep.

Recently, from 2009 onward detailed study was undertaken on Etiopathology, diagnosis and treatment of pneumonia in sheep. From lung and nasal swab, 28 Gram positive and 45 Gram negative bacterial isolated were obtained. *E. coli* and *Staphylococcus* sp. were major Gram negative and positive bacteria, respectively. The other bacteria identified were *Proteus* and *Streptococcus* sp. Three out of 13 sheep lungs were found positive for the retrovirus of ovine pulmonary adenomatosis (OPA) by U3 gene PCR.

**Johne's Disease (JD):** It is a chronic disease with no simple and reliable diagnostic tests. Clinical cases can only be detected at the advanced stage, hence it is difficult to control as the carriers can not be eliminated from the flocks. Since 1974, control of JD has been a major concern to animal health scientists. At CSWRI, Avikanagar, the overall



prevalence rate in sheep over 9 years (1978-85) was 0.7% on the basis of faecal sample examination and 10.6% on the basis of necropsy. On comparative efficacy of allergic tests, it was found that single intra dermal Johnin test was not very reliable for testing the JD. Vaccination strategy was used to control JD in sheep. Lamb-hood vaccination using the Iceland JD vaccine for a period of 7 years followed by fecal examination and elimination of positive cases from the flock, resulted in reduction of the incidence of JD from 1.26% in 1985 to 0.41% in 1987. Although fecal smear

examination can not be taken as a confirmatory and sensitive (hardly 25%) method for diagnosis of JD in sheep, it still can serve as a useful tool in JD diagnosis. As the highest clinical incidence of JD occurs around lambing and in between August and October, thus the fecal examination for detection of acid fast bacilli (AFB) may be of better use, if done during this period. During the last decade, regular screening of sheep flocks for JD exhibited that positivity for acid-fast bacilli in faecal and ICV/MLN smear was 21.76 and 63.87%, respectively, out of samples examined from clinically suspected and postmortem cases. However, the average annual positivity for JD on AGID was 5.61%. On ELISA, the seropositivity ranged from 11.42 to 21.00% with 70 to 100% cut-off values. On AGID, breed-wise analysis revealed maximum incidence in Chokla sheep (6.14%) followed by Avikalin (1.09%) and Bharat Merino (0.96%). The haematological profile showed decrease in TEC, Hb and PCV in clinical cases of JD. For effective reduction in incidence of JD through early and precise detection, protocol for PCR was standardized through targeting IS900, ISMav2 and 251 genes. In addition, for development of vaccine, organism is subjected to culture on HEYM medium with or without mycobactin J.



**Bluetongue (BT):** It is endemic in Rajasthan and a few frank cases of BT occurs in post-monsoon season at CSWRI, Avikanagar. Though mortality is low, but morbidity in the flocks have been significant including lamb losses, wool losses, congenital deformities and weak weaners and hoggets in affected flocks which causes concern. Clinical symptoms exhibited by the animals were typical as described for BT in the literature. The occurrence of cranio facial defects (agnatha, brachygna, cleft palate, bifid tongue, parrot mouth, absence of muffles, suicephalus), abnormalities of abaxial skeleton (kyphosis, torticollis arthrogyposis, tail defects etc.) shortness of legs, apodia, dwarfism were reported as congenital abnormalities in Avikanagar. The disease superimposes itself onto the breeding season causing temporary sterility in rams. The bluetongue virus (BTV) serotype 1 has also been isolated from this farm with the help of HAU, Hisar. From 1978 onwards study on epidemiology of BT was undertaken and sero-surveillance of BT was conducted covering State Sheep farms and village flocks all over Rajasthan. During 1985-88, in sheep and goats, 7.01% of the animals were found positive for BTV antibodies on AGPT. Higher prevalence (33%) of the disease was detected in exotic sheep (Rambouillet and Merino) followed by higher crosses (9%), whereas in native breed there was lack



of antibody response. Further, 3 serological tests viz. AGPT, indirect ELISA and competitive ELISA were compared for detection of BTV antibodies in sheep during 1994-96. By AGPT, 23% animals were found positive whereas ELISA could detect 63.6% positive cases. There was higher rate of sero-prevalence in exotic breeds (80.4%) than crossbreds (63.6%) and the indigenous breeds (53.6%). BTV antibodies were equally prevalent in male and female and more in hoggets than adults. Competitive ELISA and indirect ELISA were found more or less equally sensitive. As monoclonal antibody is used in competitive ELISA, thus it can be used for confirmation of doubtful cases. Dot ELISA was also standardized by using dipsticks for BTV antibodies and its results corroborated fully with indirect ELISA results. It was found to be easy and rapid and could easily be appreciated by naked eye. Therefore indirect ELISA and dot ELISA can be used suitably in detecting prevalence of BTV antibodies. Protein profile of BTV was studied by SDS-PAGE and six major proteins were analyzed in slab gel recorder.

Under All India Network programme on BT, at CSWRI, Avikanagar, to assess the sero-prevalence of BT, serum samples are being collected since 2003 covering farm and field flocks of Rajasthan state. So far a total of 11486 serum samples were deposited in serum bank maintained at divisional level. Since inception of this project, though no clinical cases of BT were observed, however, testing of serum samples using c-ELISA or indirect-ELISA revealed that the average sero-prevalence for BT ranged from 39.24% in sheep to 58.55% in goats of Rajasthan. The incidence was significantly higher (56.06%) in sheep flocks of semi-arid Rajasthan compared to 35.57% in arid Rajasthan. However, in goats there was no regional variation in sero-prevalence of BT. The management system also found to affect the sero-prevalence of BT and it was higher in migratory flocks (63.29% in sheep to 84.84% in goats) compared to stationary flocks (35.03% in sheep to 72.88% in goats). Trappers are being developed and modified to trap the *Culicoides* and other vectors responsible for transmission of BTV among livestock. In addition, attempts are also made on isolation of BT virus from suspected cases.

**Peste des Petits Ruminants (PPR):** PPR outbreaks were recorded in goat flocks of CSWRI, Avikanagar and in sheep flock of Bikaner. In Avikanagar, outbreak was recorded in a flock of 169 goats, affecting only weaners (71) and the group specific morbidity, mortality and case fatality were 40.84%, 9.85% and 24.13%, respectively. It was initially

diagnosed by clinical symptoms. Confirmatory diagnosis was made by immuno-capture ELISA by using specific monoclonal antibody. Immunoperoxidase test and fluorescent antibody test were standardized for detection of PPR antigen in various tissue samples collected from necropsied animals. At Sheep Breeding Farm, Fathepur, the sero-prevalence was 19.20% in exotic and crossbred sheep. However, in field flocks the sero-prevalence was relatively higher and ranging from 62.13% in sheep to 63.48% in goat flocks. A systematic study during the period from July 2004 to March 2006 with 2024 sheep and 1389 goats at risk in 61 flocks of 24 villages revealed that proportion of animals exhibiting typical and atypical signs of PPR was 73.77 and 27.23%, respectively. The clinical occurrence of PPR was predominantly (74%) limited to winter season. The analysis of data according to type of flock species exhibited that prevalence of PPR was maximum (50.82%) in flocks possessing only goats followed by 37.70% prevalence in flocks having mixed population of sheep and goat while minimum of 11.48% in flocks possessing only sheep. Thus, there was higher susceptibility of goats for PPR than sheep. Initially the TCRPV in use has now been replaced by specific PPR vaccine.



The division also assessed economic losses inflicted by PPR associated morbidity in flocks. The probability of outbreak of PPR in small ruminants of Rajasthan was estimated to be 50% and susceptible population of small ruminants was considered to be 45% in view of presence of PPR virus specific antibodies in 55% small ruminant population of the state. Based on livestock census (2003), the total morbidity loss due to PPR in small ruminants of the Rajasthan amount to Rs. 85.13 crore (66.86 crore in goat and 18.27% in sheep). The study revealed that PPR disease is responsible for a loss of 2.31% of total value of small ruminants (2.65% in goats and 1.58% in sheep).

**Sheep and Goat Pox:** Outbreaks of sheep and goat pox were encountered in both unvaccinated and vaccinated flocks belonging to both institute and villages since very beginning. Various vaccines from B.P. units, Jaipur, Hisar, Lucknow, Ranipet and IVRI, Iznagar were used in institute's flock to prevent the disease. Out of them, Ranipet vaccine was found to be better as compared to other contemporary vaccines as the losses in terms of diseases incidence and deaths were reasonably lower. In India sheep and goat pox



are believed to be caused by two different viruses. Hence, to our experience Ranipet SP vaccine appears to be dependable and thus recommended for control of the disease through annual vaccination by following required cold chain precautions. Specific vaccine to control goat pox is still awaited.

**Abortion:** The causes of abortion have identified to some extent. There have been abortions in ewes because of positive for chlamydial agent. Serum samples suspected for brucella are being tested regularly using coloured antigen. One field trial was conducted to determine the prevalence of brucellosis by using milk ring test in milk samples of sheep and goats. Incidence in sheep was found to be less (1.72%) as compared to goat (7.25%). In last decade, among field flocks the annual average sero-prevalence for brucellosis ranged from 2.66% in goats to 7.91% in sheep. However, with strict vigilance and monitoring for brucellosis during quarantine of newly purchased animals at CSWRI, Avikanagar, there was complete absence of the disease in both sheep and goat population at farm. During the year 2003, abortion storm was observed in Sirohi goat flock at CSWRI, Avikanagar in the month of November. The slight rise in body temperature with sudden abortion was the only clinical symptoms observed. There was severe

congestion and haemorrhage in intestine along with whitish pinpoint foci on liver of foetus. Only *E. coli* was isolated on bacterial culture.

**Subclinical Mastitis:** Detection of subclinical mastitis in sheep is difficult as compared to clinical cases which results in production losses. A total of 178 milk samples were collected from apparently healthy ewes and were subjected to bacteriological examination. Out of these, 18 were found positive for the presence of different types of bacteria with single, double or triple isolates. All the milk samples were subjected to California Mastitis Test (CMT), Whiteside Test (WST), Antitrypsin activity (ATA) and Somatic Cell Count (SCC) to determine the comparative efficacy in detecting the subclinical mastitis. The reliability of four tests in comparison to bacteriological examination was 78.2, 72.0, 90.0 and 90.0% for CMT, WST, ATA and SCC, respectively. It was also observed that ATA and SCC were more reliable and could be taken as an alternative of bacteriological examination, where large numbers of samples are to be screened or tested. Study revealed the possible role of SCM in reduction of milk yield, which in turn led to hypothermia, starvation and deaths in lambs. This needs to be studied in detail as inanition deaths in Institute flocks have been one of the major causes during 0-15 days of lambing. Prevalence of subclinical mastitis in ewes in one of the units at Avikanagar was 10.1% on animal basis.

**Enterotoxaemia:** Enterotoxaemia outbreaks recorded from the institute flocks inspite of vaccination were confirmed through laboratory testing as and when required.

**Suppurative lymphadenitis (SL)/ Caseous lymphadenitis (CL):** During 2002-03, the clinico-epidemiological profile in Sirohi goat flock at CSWRI, Avikanagar exhibited maximum incidence (66.7%) in adult followed by hogget (30.0%) and weaner (3.3%). Male had higher (72.2%) incidence compared to female (27.8%). The seasonal incidence of SL was 22.2% in monsoon, 36.7% in winter and 41.1% in summer. Though, the single / multiple abscesses occurred at parotid, pre-scapular and pre-femoral regions but most preferred sites included were pre-scapular (36.7%), pre-femoral (33.3%) and parotid gland (15.6%) area. The size of abscess varied from areca nut to cricket ball and growth was non-inflammatory. Pus samples from clinically suspected cases were directly inoculated on 5% defibrinated sheep blood agar plates. Pure colonies were prepared from the colonies after studying macroscopic characteristic of bacterial colonies, bacterial morphology and staining features by Gram's test. Colonies were inoculated on Cystine tellurite blood agar and incubated at 37° C for 72 hrs for its characteristic growth. The resultant colonies were subjected to biochemical tests like nitrate reduction, catalase, urease. Those found positive for catalase and urease, and negative for nitrate were selected for further characterization. On blood agar, a total of 17 pure *C. pseudotuberculosis* isolates were obtained. A known virulence factor, phospholipase D production was determined by detection of synergistic lysis of erythrocytes in the presence of extracellular *Rhodococcus equi* factor and hemolytic antagonism with a  $\beta$  toxin producing *Staphylococcus aureus*.



In a study involving 575 goats of Sirohi breed of CSWRI farm, overall prevalence rate of CL based on clinical, bacterial culture and PCR assay were found to be 4.7, 2.4 and 2.4%, respectively. The PCR assay developed in this study was found to be specific and rapid, and could be used for confirmation of CL in goats as an alternative method to generally cumbersome, time consuming and less reliable conventional methods.

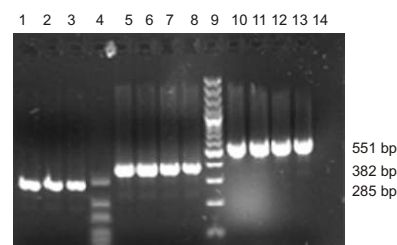
**Yersiniosis:** In goat flocks of CSWRI, Avikanagar, an outbreak of yersiniosis was recorded in the year 2005-06 with overall morbidity of 20%. The age-wise incidence was maximum (66.7%) in adult males followed by 16% in females and 10.00% in hoggets. Isolation and characterization study confirmed *Yersinia pseudotuberculosis* and organism was found sensitive to chloramphenicol, enrofloxin, levofloxin, doxycycline and gentamycin.

**Foot rot:** In the year 2010-11, outbreaks of foot rot in farm sheep and goats were investigated. The disease was observed in most virulent form in goats. The disease in sheep was however mild. After a thorough investigation, more than 20 samples from the foot rot affected animals were collected. After staining and direct microscopic examination, the causative Gram-negative bacteria indistinguishable from *Dichelobacter nodosus* and *Fusobacterium* sp. were detected, which requires confirmation. Almost all animals were successfully treated within 2-3 weeks of the outbreaks. There was no mortality; however, economic losses especially in goats were tremendous. Specific primers for molecular detection of *Dichelobacter nodosus* and *Fusobacterium necrophorum* were designed.

**Veterinary type culture:** Recently, isolates of *E. coli* and *Salmonella* were subjected to morphological and bacteriological characterization. The results on serotyping revealed existence of *Salmonella typhimurium* serotype 4,12:i:1,2. Of 16 *E. coli* isolates, 4 belonged to serogroup O66, 2 each to serogroup O60 and O95 and 1 each to serogroup O14, O20, O62, O71, O84, O87, O102 and O147. All these isolates were submitted to VTCC, Hisar. Characterised isolates of *C. pseudotuberculosis* from sheep and goats were also submitted to VTCC.

### Molecular biology

Molecular characterization of *C. pseudotuberculosis* isolated from sheep and goats were carried out. Bacterial genomic DNA extraction was made from a few colonies from the characterized /pure cultures of 48-72 hr growth on blood agar. The oligonucleotide primers synthesized from Sigma. Primers targeting the Putative oligopeptide /dipeptide ABC transporter, NADP oxidoreductase genes and Proline iminopeptidase (PIP) of *C. pseudotuberculosis* were used for PCR amplification. PCR products for sequencing were amplified with the use of Pfu DNA polymerase. PCR products were resolved on 2.5% agarose gel and bands were excised from gel and PCR products were purified using minielute gel extraction kit. Purified PCR products were sequenced by Imperial Life Sciences (Life Discoveries Pvt. Ltd.). Sequencing reports of three genes each from sheep and goat isolates were submitted to NCBI GenBank and accession numbers were received. All the 3 genes showed high degree of homology with the published sequences.



PCR amplification of DNA from *Corynebacterium pseudotuberculosis* (goat isolates). Lane 1-3 : ABC gene; lane 4: Ultra ladder; lane 5-8: NADP gene; lane 9: 100 bp ladder; lane 10-13: PIP gene and lane 14: negative control

**Gene bank submissions:** Following 6 genes were submitted to NCBI gene bank.

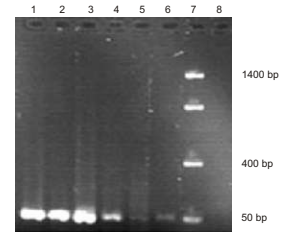
- (i) *C. pseudotuberculosis* - ABC transporter gene sheep NCBI, Gene Accession No. JF901917
- (ii) *C. pseudotuberculosis* - ABC transporter gene goat NCBI, Gene Accession No. JF901918
- (iii) *C. pseudotuberculosis* - NADP gene sheep NCBI, Gene Accession No. JF901919
- (iv) *C. pseudotuberculosis* - NADP gene goat NCBI, Gene Accession No. JF901920
- (v) *C. pseudotuberculosis* - PIP gene sheep NCBI, Gene Accession No. JF901921
- (vi) *C. pseudotuberculosis* - PIP gene goat NCBI, Gene, Accession No. JF901922

**Development of PCR assay for rapid diagnosis of CL in sheep and goats:** A PCR assay targeting three genes was developed to detect *C. pseudotuberculosis* directly from the pus samples. The assay is rapid, specific and serves as an alternative to cumbersome and time consuming bacterial culture and further biochemical characterization.

**Development of PCR assay for rapid diagnosis of JD in sheep and goats:** In view of the fastidious nature of the bacteria of Johne's disease and poor sensitivity of microscopic examination and cumbersome bacterial culture procedure, molecular detection of MAP using specific genes provides a rapid and sensitive option for specific



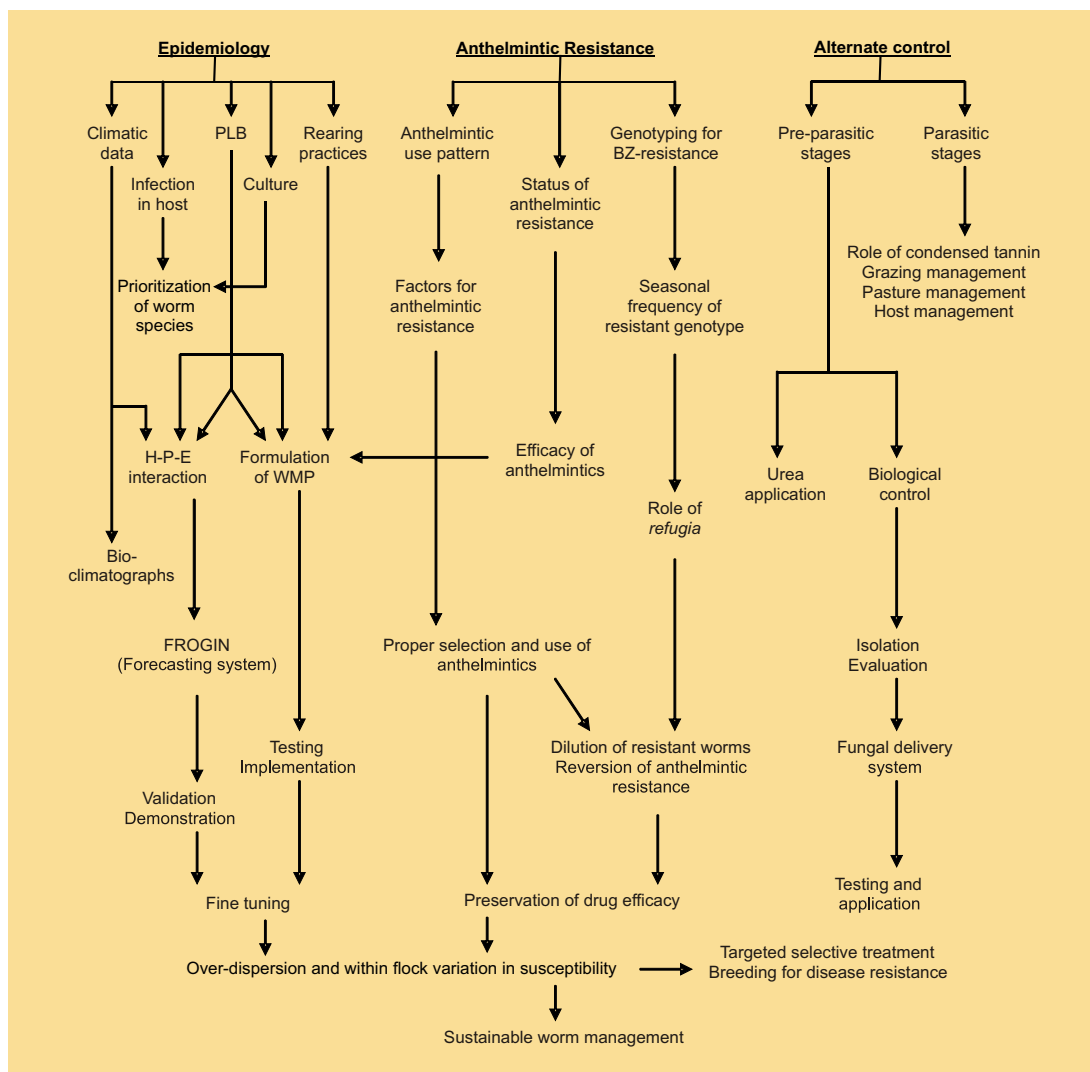
diagnosis. Genomic DNA from 54 samples (40 blood and 14 tissues) were extracted. These tissue samples were collected during post mortem of sheep and goats received from different sectors. The blood samples were collected from Chokla sheep. The PCR protocols targeting IS900, ISMav2 and 251 genes were standardized on positive samples and were subsequently applied on test samples. PCR was applied on DNA extracted from 40 blood samples. Three samples each yielded positive amplifications with ISMav2 and 251 genes. Tissues of 15 JD suspected cases collected at necropsy were subjected to 251 gene PCR, of which 13 were detected positive for MAP genome.



PCR amplification of 251 gene. Lane 1: positive control; lane 2-6: test samples; lane 7: 50 bp ladder; lane 8: negative control

### Parasitology

In the field of Parasitology, during initial phase mainly need-based research work was carried out. However, with the emergence of problem of anthelmintic resistance in sheep nematodes, the systematic work on epidemiology at farm level was initiated in 1994 and later on the entire Rajasthan state was covered under All India Network Programme on Gastrointestinal Parasitism. The basic road map considered for research is depicted below:



**Epidemiological studies:** The results of initial studies at farm exhibited that intensity of strongyle infection in adult sheep started rising from April and reached a peak in August and then declined to a very low level until March. In young sheep, intensity of infection remained nil up to June and began to rise from mid July to reach a maximum in early September. The availability of infective larvae on herbage was mainly restricted for the period from July to October. The predominant nematode parasite of economic significance at farm is *Haemonchus contortus* followed by *Oesophagostomum columbianum*, *Trichostrongylus axei* and *T. colubriformis*. The worm burden in sheep exhibited the positivity of abomasi for *H. contortus* and *T. axei* infection to the tune of 91.7%. Of these 83.2% cases were infected with only *H. contortus* while 16.7% were infected with mixed infection of both. The average worm count varied from nil (February) to 7694.4 (August). The young animals up to one year of age harbored more worms compared to adults. The observations on development and survival of larvae on pasture revealed that infective larvae survived for maximum period (up to 9 weeks) during September followed by August and November (8 weeks). The study indicated that in semi-arid condition contaminated pasture should be kept free from grazing at least up to 9 weeks to make it parasitologically safe pasture for grazing. On the basis of above findings deworming frequency at CSWRI, Avikanagar was reduced from 4 times a year to once a year (with one tactical drench). This not only economized the worm control programme but also decrease the selection pressure on parasites population needed to develop anthelmintic resistance thus extended the life of existing anthelmintics.



Since 2000, with the launching of All India Network Programme on Gastrointestinal Parasites, extensive study on epidemiological aspect was undertaken in both arid and semi-arid agroclimatic conditions of Rajasthan involving flocks managed at farm or field level. The major findings are as follows:

**Anthelmintic use pattern in Rajasthan:** In field flocks of Rajasthan use of anthelmintics is the only measure adopted to control problem of gastrointestinal parasitism in sheep. The mean annual frequency of strategic anthelmintic drench was  $2.59 \pm 0.03$  / sheep / year. In majority of flocks (51.9%) the animals received 3 drenches of anthelmintics in a year followed by 2 drenches / year (39.9% flocks). The common drenching period were during April / June, September / November and February indicating that practice of anthelmintic intervention is coupled with shearing of sheep. Benzimidazole (BZ) and tetraisoazole (TEM) group of anthelmintics are commonly used by farmers. Both of these anthelmintics were used by >50% of farmers regularly since their introduction into the market. Only 13.0 and 12.4% farmers used single class of anthelmintic either BZ or TEM, while, 19.2% of farmers used many other types of anthelmintics (rofoxanide, closantel, CUNIC mixture etc) in addition to BZ and TEM.

**Incidence pattern of strongyle worms in sheep:** The overall annual incidence of strongyle worms in sheep flocks remained around 55.0% and 65.0% in field and farm flocks, respectively. A marginally higher incidence was noticed in semi-arid conditions under both the management systems. The monthly incidence exhibited that among farm flocks the incidence was higher (> 65.0%) from May to November in semi-arid region and from July to November and April in arid region of Rajasthan. Similarly in field flocks >65% incidence was observed from June to October in both the agroclimatic regions. In all types of management and agroclimatic conditions, the seasonal incidence was highest (70-80%) during monsoon followed by summer and lowest during winter.

**Intensity of strongyle worms in sheep:** In semi-arid Rajasthan, at an organized sheep farm the mean monthly FEC varied from  $128.37 \pm 9.56$  (February) to  $1753.10 \pm 125.36$  (July) eggs per gram (epg) of faeces. The intensity of infection remained higher from July to September only. The initial rising trends in FEC occurred in the month of June, probably due to resumption of development of hypobiotic worms with in the host, giving peak of infection in July and provide the source of pasture contamination during monsoon. The decline in FEC in the following months may be due

spontaneous occurrence of self-cure phenomenon following acquisition of fresh wave of infection from pasture and expulsion of old worms.

In contrast to farm flocks, the intensity of strongyle infection remained lower in the field flocks. The mean monthly FEC ranged from  $87.65 \pm 5.50$  (March) to  $1012.50 \pm 39.20$  (September) epg. Similar to farm situation in field flocks the FEC started rising from June onward and reached to peak at the end of monsoon. The magnitude of FEC during the period from November to May revealed that there is occurrence of hypobiotic phenomenon and absence of periparturient rise (PPR) in field flocks also. The frequency distribution of FEC data suggested that during the months favourable for parasitic propagation on pasture, around 20-30% of the animals in flock had > 1000 epg, otherwise in rest of the year majority of animals are either free from infection or had very low FEC.

In arid Rajasthan, the mean monthly FEC in farm flocks ranged from  $194.39 \pm 11.81$  (March) to  $2314.64 \pm 221.17$  (October) epg. The intensity of strongyle infection showed higher trend during monsoon and post-monsoon period. Within flock the distribution of FEC data exhibited that around 40% of animals had intensity to the tune of >1000 epg during July to October. Comparatively higher proportion of animals found to possess >1000 epg during winter months (November to February) in arid farm than semi-arid farm and it could be attributed to higher prevalence of *Trichostrongylus* spp. in arid region and presence of favourable climatic conditions for translation of exogenous stages of *Trichostrongylus* spp. Similar to semi-arid region, the comparison of FEC between farm and field flocks exhibited that the intensity of strongyle infection remained relatively low in field flocks. The mean monthly FEC varied from  $59.20 \pm 5.80$  (April) to  $1429.22 \pm 50.45$  (August) epg. The FEC pattern revealed declining trend after single peak of infection during August-September. The magnitude of FEC during the period from November to May exhibited that there is occurrence of hypobiosis and absence of PPR in flock of this region also. During peak favourable season (July-September) around 30% of animals found to possess >1000 epg, otherwise in rest of the months majority of sheep are either free of infection or possess very low level of infection.

*Peri-parturient rise (PPR) in faecal egg count:* In a study conducted at CSWRI, Avikanagar during spring lambing season it was observed that there was no significant difference in FECs between lactating and dry ewes up to 12 weeks post lambing as well as FECs remained almost similar in sheep maintained under different stages of reproduction. The studies on lambing pattern in field flocks of Rajasthan revealed that majority of ewes in flocks are in lambing / lactation during October to February and agroclimatic conditions of the state does not favour the development and dissemination of worms from December to June resulting in non-availability of source of infection for lactating ewes. On the basis of these studies it was concluded that in Rajasthan there was absence of classical PPR in FEC in sheep. However, this phenomenon appeared to occur in ewes which lambed during July-August (favourable season for peak parasitic activity on pasture).

*Pasture infectivity pattern:* In Rajasthan under different agroclimatic and management system, the overall annual pasture infectivity ranged from  $203.5 \pm 72.9$  in arid farm to  $363.8 \pm 85.6$  L<sub>3</sub> / Kg DM of herbage in semi-arid farm. In both the agroclimatic regions and management systems higher pasture larval burden (PLB) occurred during monsoon season except in arid field where it was marginally higher during summer. The small peak in PLB during February is attributed to favourable environmental as well as resumption of hypobiotic worms and increased contamination of pasture by faeces from ewes in lambing. The PLB started rising with pre-monsoon shower and reached to peak in August. On the contrary, the pasture infectivity on community grazing land (CGL) in semi-arid region was mainly restricted to monsoon season with a single peak in the month of July. In arid agroclimatic region, the PLB started rising from mid-monsoon and peaked in the month of February in farm area.

*Generic composition of strongyle larvae on coproculture:* The commonest species recorded was *H. contortus* followed by *Oesophagostomum*, *Trichostrongylus axei* and *T. colubriformis*. The annual prevalence of *H. contortus*

larvae on coproculture ranged from 55.5% (semi-arid) to 67.8% (arid) in farm flocks and from 89.5% (arid) to 96.8% (semi-arid) in field flocks. The seasonal variation in proportion of *H. contortus* larvae was clearly evident in farm flocks with maximum during monsoon season. The monthly proportion varied from 24.4% (March) to 83.1% (September) in semi-arid farm, 44.5% (January) to 83.7% (August) in arid farm, 92.1% (April) to 99.6% (August) in semi-arid field and from 83.7% (March) to 98.3% (August) in arid field.

The annual proportion of *O. columbianum* larvae on coproculture varied from 13.7% (arid) to 20.5% (semi-arid) in farm flocks and from 2.3% (semi-arid) to 4.1% (arid) in field flocks. In general seasonal pattern revealed higher prevalence of *O. columbianum* larvae during summer in both the management and agroclimatic conditions followed by winter and minimum during monsoon. The monthly proportion of *O. columbianum* larvae on coproculture ranged from 5.6% (September) to 35.6% (April) in semi-arid farm, 2.2% (August) to 24.1% (March) in arid farm, 0.3% (August) to 7.4% (April) in semi-arid field and from 0.1% (October) to 17.1% (April) in arid field.

The annual proportion of both *T. axei* and *T. colubriformis* on coproculture varied from 18.5% (arid) to 24.0% (semi-arid) in farm flocks and from 0.9% (semi-arid) to 6.4% (arid) in field flocks. Relatively higher prevalence was noticed during winter except in semi-arid farm. The monthly prevalence ranged from 8.4% (July) to 51.0% (March) in semi-arid farm, 9.0% (June) to 36.9% (January) in arid farm, nil (March) to 2.1% (September) in semi-arid field and from 1.0% (April) to 14.7% (March) in arid field.

**Epidemiology of *Trichuris* spp.:** The overall annual incidence of *Trichuris* spp. in sheep flocks of Rajasthan varied from 0.5% (arid farm) to 1.6% (semi-arid field) revealing marginally higher incidence in semi-arid agroclimatic conditions in both farm and field flocks. Though, there was no distinct seasonal variation in the incidence rate, however higher incidence was observed in winter season (after hot-dry weather). The monthly incidence ranged from nil (March) to 3.7% (November) in semi-arid farm, from nil (July) to 1.4% (December) in arid farm, from 0.5% (October) to 3.1% (January) in semi-arid field and from 0.2% (June) to 2.2% (September) in arid field.

**Epidemiology of *Strongyloides papillosus*:** The farm flocks had significantly higher prevalence (11.9-13.6%) of *S. papillosus* than field flocks (0.9-2.9%) in both the semi-arid and arid agroclimatic conditions. The overall annual incidence ranged from 0.9% (arid field) to 13.6% (semi-arid farm). The monthly incidence ranged from 6.0 (February) to 36.2% (August) in semi-arid farm, from 2.7 (May) to 22.3% (September) in arid farm, from 0.5% (February) to 6.6% (September) in semi-arid field and from nil (May) to 2.4% (July) in arid field.

**Epidemiology of *Fasciola gigantica*:** The study carried out in arid and semi-arid Rajasthan revealed an overall incidence of *F. gigantica* in sheep flocks to the tune of 0.2 and 0.1%, respectively, on faecal examination. The monthly egg shedding pattern exhibited higher incidence in June (semi-arid region) and February (arid region) indicating possible period of ingestion of metacercariae during March in the vicinity of snail habitats in semi-arid Rajasthan. In arid region, migratory sheep found to pick up infection during November-early December and in May-June during their grazing around water reservoirs. The seasonal analysis showed higher prevalence of infection during summer in semi-arid region compared to during winter in arid region.

**Epidemiology of stomach flukes:** The faecal examination from sheep flocks revealed significant variation in the prevalence of paramphistomes among both the agroclimatic conditions of Rajasthan. The overall annual incidence of stomach flukes in sheep ranged from 1.6% in arid Rajasthan to 7.5% in semi-arid Rajasthan. The monthly rate of prevalence also exhibited significant variability and ranged from nil (December) to 31.7% (August) in semi-arid region and from nil to 7.9% (August) in arid region. In semi-arid conditions it has been observed that infection is endemic in nature with two peaks of in a year during the month of June and August compared to only a single peak during August in arid conditions of Rajasthan.

**Epidemiology of Schistosomes:** Among sheep flocks of Rajasthan, the annual incidence of *S. indicum* (on the basis of faecal examination) was 0.3 and 0.5%, respectively, in arid and semi-arid regions. In semi-arid region the monthly incidence ranged from nil (January) to 1.9% (June) compared to almost nil (November-April) to 1.6% (August) in arid region. The peak / higher incidence occurred during May to September in semi-arid and during August in arid region regions.

**Epidemiology of cestodes:** *Moneizia expansa* and *M. benedeni* are commonly encountered. The overall annual incidence of *Moniezia* infection in sheep flocks of Rajasthan ranged from 4.0 to 5.8% in semi-arid and from 0.7 to 1.8% in arid region. The monthly incidence on faecal examination showed seasonal variability in occurrence particularly in semi-arid Rajasthan. Among farm animals of semi-arid region higher incidence was observed during May to August. On retrospective study on the occurrence of cestode infection at CSWRI, Avikanagar it was presumed that probability for acquiring infection was higher during winter because of practice of feeding beri-pala leaves (contaminated with oribatid mites) in late winter to animals. In field flocks of same region the monthly incidence ranged from 3.4% (July) to 8.3% (December), revealing two peaks during August and December.

**Epidemiology of coccidia:** The species recorded were *Eimeria arloingi*, *E. ovina*, *E. faueri*, *E. parva*, *E. granulosa*, *E. intricata*, *E. pallida* and *E. ninakohlyakimovae*. Of these *E. ninakohlyakimovae*, and *E. ahsata* are most pathogenic to lambs (1-6 month of age). *E. faueri*, *E. arloingi*, *E. granulosa* and *E. parva* are mild pathogenic while *E. intricata* is usually non-pathogenic in sheep. In Rajasthan the overall annual incidence of *Eimeria* infection in sheep flocks managed under farmer's condition ranged from 41.8 (arid) to 44.5% (semi-arid). In organized farm the annual incidence varied from 30.1 (arid) to 37.5% (semi-arid). The monthly incidence on faecal examination showed seasonal variability in incidence of *Eimeria* infection. In all the agroclimatic and managerial conditions maximum incidence was noticed during monsoon (34.28 to 49.73%) followed by winter and minimum in summer (25.21 to 35.79%).

### **Anthelmintic resistance**

The original optimism of successful control of GI parasites with novel broad spectrum anthelmintics received a setback with emergence of resistant strains of parasites. It frustrates worm control measures and limits the available anthelmintic types to combat the primary problem of worms. Failure of benzimidazole anthelmintic at CSWRI, Avikanagar was first suspected during 1993. The detailed investigations were carried out to detect anthelmintic resistance in nematode parasites during 1994. Both *in vivo* and *in vitro* tests were carried out to detect the emergence of anthelmintic resistance.

**Benzimidazole (BZ) resistance:** On *in vivo* faecal egg count reduction test (FECRT), the efficacy for fenbendazole and tetramisole was more than 95% while for albendazole it was 80% at CSWRI, Avikanagar. Pre and post- treatment faecal culture revealed that *H. contortus* had developed resistance to BZ. Further the isolated strain of *H. contortus* revealed LC<sub>50</sub> value of 0.239±0.13 µg TBZ/ml on EHA. Larval development assay (LDA) using commercially available kit was performed to confirm the results, which supported the results obtained by FECRT and EHA. More over the LDA data also revealed the emergence of BZ-resistant *Trichostrongylus* spp., which could not be detected on FECRT because of low proportion of their larvae on coproculture. The results also indicated that there was no multiple resistance against BZ/LEV in *H. contortus* at this farm as the combination of BZ/LEV gave 100% efficacy on LDA.

In field flocks around Avikanagar, emergence of BZ resistant *H. contortus* was recorded with efficacy of 64-88% against fenbendazole in FECRT. On EHA the LC<sub>50</sub> of TBZ against *H. contortus* ranged from 0.157±0.016 to 0.493±0.053 µgTBZ/ml. Due to failure of anthelmintic treatment at Large Scale On Sheep Breeding Farm, Fatehpur (Sikar) in arid region of Rajasthan, FBZ was found to have nil efficacy against *H. contortus*. The EHA showed LC<sub>50</sub> value of 0.625±0.098 µg TBZ/ml.

**Rafoxanide (RFX) resistance:** In flocks naturally infected with *H. contortus* at CSWRI, Avikanagar, the reduction in FEC with RFX was 31% with 0 lower 95% confidence limit suggested emergence of resistance to RFX in *H. contortus*. At LBSSF, Fatehpur, the efficacy of RFX against *H. contortus* was 96% with lower 95% confidence limit less than 90 suggested suspected resistance to RFX.

**Tetramisole (TEM) / Levamisole (LEV) resistant:** The resistance to LEV in *H. contortus* was detected only at LBSSF, Fatehpur, where its efficacy was 25%. At CSWRI, Avikanagar, the % efficacy of LEV against *H. contortus* was 96% with lower confidence limit less than 90 which indicated suspected resistance against the LEV. The results were also confirmed by LDA, which showed 97% efficacy. However, in animals of ARC, Bikaner the LEV was found 100% effective against *H. contortus*.

As *H. contortus* was the predominant parasite at CSWRI, Avikanagar and LBSSF, Fatehpur and had developed resistance against major anthelmintic group (BZ), replacement of this class of anthelmintic with other narrow spectrum anthelmintic (Closantel) was advocated. This change has yielded effective control of GIN in sheep.

**Prevention of borrowing of anthelmintic resistant parasites:** Regular monitoring for status of anthelmintic resistance in animals introduced in CSWRI, Avikanagar from outside is being performed in quarantine. Indian Karakul transported from Arid Region Campus, Bikaner to CSWRI, revealed that FBZ was 100% effective in reducing the egg count on FECRT. LC<sub>50</sub> value on EHA was 0.074±0.015 µg TBZ/ml. It was concluded that the Bikaner strain of parasite was susceptible to BZ. Presence of BZ and TEM susceptible strains of strongyle worms was encountered in Garole sheep from West Bengal. Patanwadi sheep from Gujarat found to possess TEM resistant and BZ susceptible strains while Kendrapada sheep from Orissa exhibited presence of BZ resistant strains of strongyle worms. Suitable measures were undertaken to prevent the entry of resistant worm at CSWRI, Avikanagar.

**Extent of anthelmintic resistance in Rajasthan:** Under All India Network Programme on GI Parasitism, state-wide prevalence of anthelmintic resistance in nematode parasites was studied in sheep flocks by employing both *in vivo* FECRT and *in vitro* EHA. The overall BZ-resistance in *H. contortus* was observed in 85.7% of flocks in Rajasthan. TEM-resistant strain of *H. contortus* was observed in 56.5% of the flocks in Rajasthan. On *in vitro* EHA, BZ-resistance was detected in 73.0% of the flocks tested with the overall mean ED<sub>50</sub> value of 0.200±0.008 µg TBZ/ml. It was interesting to observe the seasonal variation in efficacy of FBZ which indicated variation in population dynamics of parasite and proportion of susceptible and resistant parasites in host. The mean efficacy (%) for FBZ ranged from 56.80±3.99 to 79.76±2.23 with 94.4% and 81.1% of the flocks tested to possess BZ-resistant population of *H. contortus* during the period from June to August and December to February, respectively. The proportion of *H. contortus* in host was inversely related to the efficacy of FBZ. The % efficacy of TEM in field flocks of Rajasthan ranged from 72.65±6.83 (March-May) to 65.46±2.07 (December-February). The low efficacy and higher prevalence of TEM-resistant parasites (70.6% of the flocks) during March to May, indicated that both *H. contortus* and *Oesophagostomum* spp might be resistant to TEM, while higher efficacy and minimum prevalence of TEM-resistant parasites (46.4% of the flocks) during December to February suggest that *Trichostrongylus* spp were susceptible to TEM. The overall mean % efficacy of CLS against *H. contortus* was 99.43±0.16 throughout the year.

**Variation in LC<sub>50</sub> value during patency of infection:** During the patency of *H. contortus* infection in sheep the LC<sub>50</sub> values of TBZ on EHA showed parabolic pattern in both resistant and susceptible strains. The maximum LC<sub>50</sub> value was observed between 40-50 days post-infection. However, LC<sub>50</sub> value for susceptible strain never reached to the level of 0.1 µg TBZ/ml that is the value used to discriminate susceptible and resistant strain. Like-wise the LC<sub>50</sub> values always remained > 0.1 µg TBZ/ml for resistant strain. It was concluded that day to day variation in LC<sub>50</sub> values during patency of infection on EHA does not alter the diagnostic interpretation and EHA can be used a suitable diagnostic test.

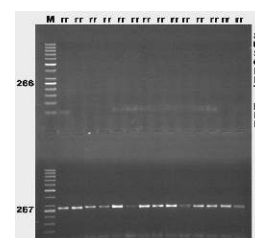
**Standardization of larval development assay (LDA):** Nematode eggs were placed into the wells of micro-titre plates and hatched larvae developed to the L<sub>3</sub> stage in the presence of anthelmintic. During standardization process concentration of eggs, nutritive medium and volume of eggs were undertaken. Nutritive medium @ 5-10% of egg suspension yielded optimum development of L<sub>3</sub>. Combination of EBSS (1 part) and Yeast extract (9 parts) as nutritive medium gave maximum L<sub>3</sub> development. Minimum contact of eggs with saturated salt solution during their separation from faeces yielded best results. Concentration and volume of egg suspension were found to have non-significant effect on L<sub>3</sub> development. On incorporation of anthelmintic (TBZ) in agar matrix, it was observed that the mean L<sub>3</sub> development in susceptible strain varied significantly from 61.92 to 77.09% in wells containing nil to 0.078 µM TBZ / ml. In susceptible strain L<sub>3</sub> development was significantly inhibited in more than 50% of eggs with concentration of TBZ > 0.078 µM / ml. On the other hand, with resistant strain more than 50% eggs were developed to L<sub>3</sub> at concentration ranged from nil to 2.50 µM TBZ/ml, revealing around 30 times higher concentration of TBZ required inhibiting L<sub>3</sub> development in resistant strain compared to susceptible strain.

**Factors affecting the rate of development of anthelmintic resistance:** In a state-wide survey in Rajasthan it was observed that flock size had no significant influence on drench frequency in the flock. A linear increase in prevalence rate of anthelmintic resistance was observed with increase in flock size. On FECRT, it was observed that emergence of BZ and TEM resistance in *H. contortus* was significantly influenced by drench frequency and had positive correlation. Though flock size had no direct influence on extent of AR but indirectly it plays its role through use of more number of drenches in larger flocks.

A study was conducted to observe the seasonal variation in different genotypes of *H. contortus* (w.r.t. BZ-resistance) and possible role of *refugia* in development of AR. The PCR based analysis revealed that in adult male *H. contortus* (from CSWRI, Avikanagar), the overall annual frequency of rr, rS and SS types was 72.5, 25.5 and 2.0, respectively. Seasonal analysis revealed that sheep harboured more number of BZ-susceptible worms during monsoon and winter compared to summer.

The variations in genotypic frequency were also studied using infective larvae (L<sub>3</sub>) of *H. contortus* from different sources. In semi-arid farm, with respect to BZ-resistance the overall annual frequency of rr, rS and SS types of *H. contortus* larvae recovered from sheep and found to contribute to gene pool of supra-population on pasture was 86.38, 13.19 and 0.43, respectively. Further on monthly analysis of the results, it was observed that prior to strategic anthelmintic intervention, the proportion of rr genotype in gene pool of larvae was > 90% in host (up to September). Following deworming in mid-September the proportion of rr, rS and SS genotypes was 40.0, 55.0 and 5.0%, respectively. In succeeding months when environment is not favourable for dissemination of pre-parasitic stages of parasite with minimal chances of reinfection, the ratio of different genotypes in supra-population returned to previous level with >95% of rr genotypes. The seasonal pattern revealed relatively higher prevalence of BZ-susceptible genotypes in supra-population during monsoon season.

At arid farm, the overall annual frequency of rr type of *H. contortus* larvae recovered from sheep and found to contribute to gene pool of supra-population on pasture was 59.41% and varied from 49.50% (winter) to 73.56% (summer). On monthly basis, the frequency of rr genotype ranged from 40.0% in August to 100.0% in July. The relatively higher proportion of rr genotype in supra-population during the period from March to July indicates existence of unsuitable climate for larval dissemination as well as meager chances of reinfection from pasture. In comparison to semi-arid farm there was higher prevalence of BZ-susceptible *H. contortus* larvae in supra-population.



AS-PCR profile of beta-tubulin isotype 1 gene of *Haemonchus contortus* infective larvae

In field flocks of semi-arid Rajasthan the proportion of rr genotype of *H. contortus* larvae found to contribute to gene pool of supra-population on pasture was 97.58, 88.57 and 96.43%, respectively during summer, monsoon and winter season. It has been observed that in pre-drench gene pool the frequency of rr genotype was 92.5% which decreased slightly to 75.0% in October indicating that smaller proportion of susceptible genotypes able to disseminate in later half of monsoon and dilute the gene population on refugia. Thus, deworming in later half of monsoon is helpful in increasing the efficacy of anthelmintics by increasing the size of refugia. Rising trend in prevalence of rr genotype from November onwards might reflect the role of migration and unplanned grazing and anthelmintic use in field flocks in maintaining the high level of BZ-resistance. In Arid field, the proportion of rr genotype of *H. contortus* larvae found to contribute to gene pool of supra-population on pasture was 85.62, 86.54 and 92.13%, respectively during summer, monsoon and winter season. Similar to semi-arid region, it has been observed that in pre-drench gene pool the frequency of rr genotype was around 90.0% which reduced to 40.0% in October but reached to pre-drench level thereafter.

The correlated response between genotypic frequency of homozygous BZ-resistant larvae and BZ-efficacy exhibited that a marginal rise in the proportion of BZ-susceptible alleles during September to November resulted in increase in efficacy of BZ anthelmintics as well as reduces the proportion of flocks harbouring BZ-resistant *H. contortus*. It is clear from the molecular analysis that community dilution strategy for reversion of susceptibility to BZ in *H. contortus* could be feasible in farm condition. The period from September to November in semi-arid and from June to February in arid Rajasthan was found appropriate in increasing the frequency of BZ-susceptible alleles in the refugia.

### Studies on pharmacokinetics of benzimidazole in Sheep

The studies were undertaken in collaboration with NDDB, Anand (Gujarat) with the objective to manipulate the pharmacokinetics of benzimidazole in an attempt to extend the life of existing BZ anthelmintics and to evaluate the preventive medication by targeting more susceptible larval population of parasite nematodes through long-term low-level medication incorporated in feed pellets.

*Effect of route of administration of ABZ in sheep:* It was concluded that oral administration of anthelmintic in ruminants stimulated the closure of oesophageal groove leading to diversion of drug to the abomasum resulting in faster absorption and elimination. Thus, the increased bioavailability of BZ anthelmintics given through intraruminal route could be exploited for optimizing the use of anthelmintic for sustainable parasite control.

*Effect of diet type on the pharmacokinetics of ABZ in sheep:* The effects of changing the proportion of two locally available green and dry fodders in the diet of sheep on the disposition of ABZ metabolites in plasma revealed significantly higher plasma concentration of ABZ-SO<sub>2</sub> following 2 hr of ABZ administration in mixed and dry fodder group compared to green fodder fed group. The ABZ-SO concentration remained significantly higher in dry group at 10-12 hr of administration (1.914±0.166 to 1.977±0.104 µg/ml) compared to other groups. There was a substantial reduction in the bioavailability of both the metabolites in sheep offered fresh green fodder compared to those maintained on dry mature hay. Animals offered dry fodder had the higher mean C<sub>max</sub>, T<sub>max</sub>, AUC and MRT value compared to other groups suggesting higher uptake, greater availability of the drug for absorption and slower elimination. It was concluded that the decreased transit time of digesta on the green fodder reduced systemic availability by reducing the time available for gastrointestinal absorption of the drug.

*Effect of pre-treatment fasting on anthelmintic availability in sheep:* The study suggested that restricting the intake of feed is an alternative means of enhancing the systemic availability by increasing its dissolution and absorption and delaying the elimination of anthelmintics. Thus, allowing more time to contact between drug and parasites. Restriction of feed intake may be practiced to enhance the anthelmintic efficacy against parasites.



*Efficacy of long-term low-level intraruminal ABZ in sheep experimentally infected with BZ-resistant H. contortus:* The result revealed that on long-term low-level dosing with ABZ, the ABZ metabolites reached plateau level after 4 day and remained almost unchanged up to day 10-11 indicating stage of equilibrium between the absorption and disposition of drug by day 4. In comparison to single intraruminal administration (@ 5.0 mg/Kg), long-term low-level administration (@ 0.5 mg/Kg/day for 10 days) resulted in lower mean  $C_{max}$  and longer  $t_{1/2}$  value. The higher AUC suggested more period of parasite-drug contact at required level in long-term low-level approach. The FEC and abomasal worm count revealed that though, a plateau concentration of ABZ metabolites reached, but it was unable to kill the incoming larvae, however, reduced the fecundity of parasite. The study suggested that long-term low-level medication of albendazole against BZ resistant worm failed to reduce establishment of incoming larvae but showed effect against egg laying capacity of adult worms, resulting in low egg output. Thus, it may be concluded that medication in divided doses against resistant worm might be helpful in reducing the pasture contamination.

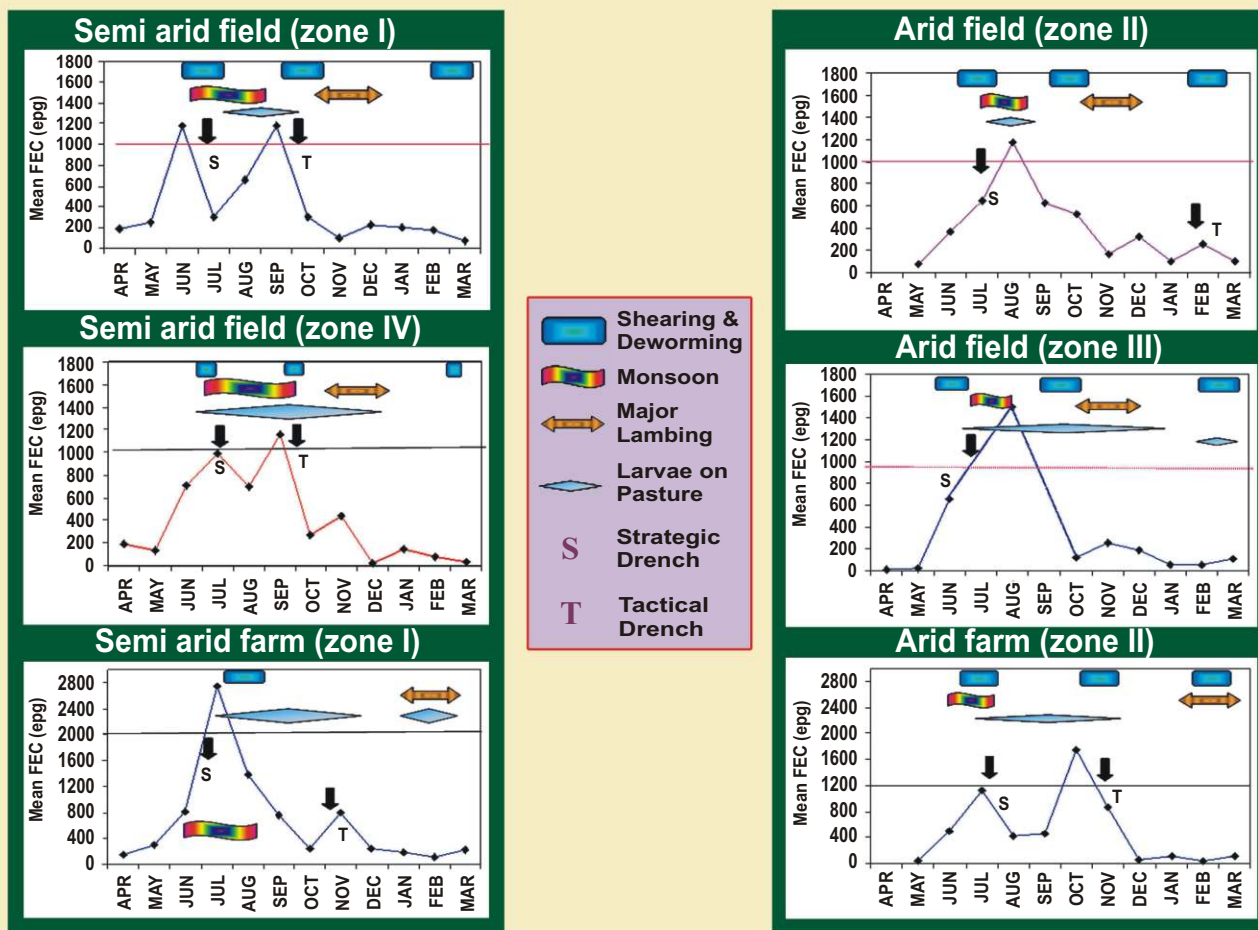
*Efficacy of long-term low-level anthelmintic medication through feed pellets:* Long-term low-level delivery of ABZ through feed pellets could successfully control the nematode parasites in hoggets. The faecal egg count (FEC) in group receiving medicated feed pellets (MFP) remained at low-level upto 3 weeks post treatment. Similarly single oral treatment with Nilverm (TEM) also reduced FEC upto 3 weeks PT but thereafter, there was significant rise in FEC in comparison to MFP group. On the other hand single oral treatment with closantel (CLS) due to its protein binding capacity controlled the intensity of infection upto 8 weeks PT, where the FEC remained <100 epg. Following long-term low-level administration of ALB in feed pellets the plasma disposition profile of the metabolites explained three distinct phases, a first order absorption and adaptation for 5 days, plateau from day 5 to 30 and first order elimination from day 31 to 33. The AUC value of ABZ-SO of more than 1000  $\mu\text{g hr/ml}$  and long elimination half-life could readily kill the incoming infective larvae and prevent their establishment in the abomasum. It was concluded that CLS and medicated feed pellets could be used for sustainable parasite control in sheep at epidemiologically strategic points, so that incoming larva from pasture is killed before establishment.

### **Worm management programme**

Based on epidemiological investigations, region based modified worm management programme (MWMP) for heep flocks of Rajasthan has been developed and tested for their effectiveness. It involves single targeted anthelmintic treatment of flocks during mid to late monsoon with tactical treatment (if required) in November. The monthly FEC pattern revealed that drenching of flocks during mid to end phase of monsoon able to prevent build up of infection in succeeding months in contrast to early monsoon drenching. Further unfavourable climatic conditions, denudation of grass cover / mat in grazing area, practices of grazing in harvested field or on top feeds resulted in significant decline in FEC in succeeding period without any anthelmintic intervention. The farmer's practice of 2-3 drench / year seems to be unwarranted as there is continuous decline in FEC after monsoon. Therefore, proposed MWMP based on interaction between epidemiology, weather and management of flocks resulted into reduction in drench frequency, allows better management / rotation of anthelmintic types, reduces selection pressure in parasite population, delayed emergence of anthelmintic resistant strains of parasites, extend life of existing anthelmintics and reduces the unwanted expenditure incurred by farmer on anthelmintic use. Further study revealed that early deworming during July is not fruitful, hence it should be delayed till infection reaches to peak (particularly after mid monsoon) so there could be large population on *refugia* which maintain the balance between different genotypes (w.r.t. anthelmintic resistance) in population.

*Flock performance and economic impact of MWMP:* The annual lambing rate remained higher (83.40%) in flocks managed through MWMP compared to flocks maintained by TWMP (78.21%). The annual morbidity in field flocks varied from 42.21% (TWMP) to 43.18% (MWMP). The annual mortality ranged from 6.49 (arid) to 10.26% (semi-arid) in flocks drenched once a year. A marginally higher mortality (7.06-11.99%) was noticed in flocks drenched 2-3 times

in a year. The morbidity and mortality pattern revealed non significant influence of reduced deworming in both the agroclimatic regions. The annual disposal rate through sale of animals (particularly lambs and old aged ewes) remained almost similar (18.05% in TWMP to 20.08% in MWMP) in both the system of worm management. The total average expenditure per 100 sheep per year was Rs. 28330.0 in flocks drenched according to MWMP compared to Rs. 24250.0 in conventionally drenched flocks. The average annual net income per 100 sheep per year varied from Rs. 55805.0 (arid) to Rs. 69388.0 (semi-arid) in flocks with MWMP compared to conventionally drenched flocks where it ranged from Rs. 50806.0 (arid) to Rs. 53175.0 (semi-arid). Similarly at farm level, performance was not hampered at any stage in flocks maintained on single drench regime compared to conventional >2 drench / year schedule.



**Modified worm management programme for sheep flocks of Rajasthan**

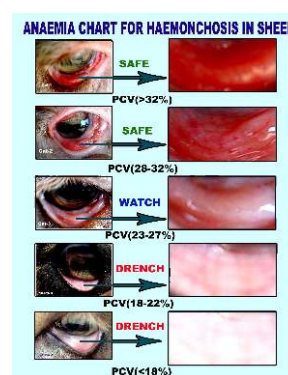
*Evaluation of flukicide intervention in sheep flocks of semi-arid Rajasthan:* Based on epidemiological observations, it was realized that in semi-arid region of Rajasthan among flukes only the amphistomosis had endemicity and require a suitable intervention. Keeping in mind the incidence pattern of amphistomes and their life cycle, flukicide intervention (Oxyclozanide) during the period from end of March to mid April was applied and tested for its effectiveness in curtailing the incidence rate. The monthly incidence rate for *Amphistomum* ova in faecal sample from un-drenched

animals varied from nil (December) to 32.42% (August) with an overall annual incidence rate of 7.89%. On the other hand in samples from drenched flocks the monthly incidence ranged from nil (November to February) to 8.84% (July) with overall annual incidence to the tune of 2.21%. The strategic inclusion of flukicide (to overcome the immature amphistomosis) during March-April exhibited significant reduction (~70%) in incidence of amphistomes.

### Worm management by targeted selective treatment (TST) in sheep flocks

Because of rapid emergence, spread and borrowing of anthelmintic resistance in *H. contortus* and limited option for selecting a suitable anthelmintic class an attempt was made to develop the suitable strategy for application of TST approach in sheep flocks. Following successful implementation of single drench / annum schedule during mid to late monsoon, it was planned to further reduce the use of anthelmintics in flock to harvest the benefits of over-dispersion phenomenon in FECs.

**Magnitude of FEC, PCV and Hb in visually anaemic and normal sheep:** The mean FEC remained significantly higher (~ 3 times) in visually anaemic group compared to visually normal group during wormy season (August-November). However, in non-wormy season (December-March) the intensity of strongyle infection remained at acceptable level (< 500 epg) in both the groups. The mean PCV ranged from 18.50±0.50% (March) to 27.20±1.24% (August) in visually anaemic group and from 24.67±0.65% (March) to 31.84±0.71% (October) in visually normal group indicating significantly lower values in anaemic group during both wormy and non-wormy season. Similar pattern was observed for haemoglobin. The correlation between FEC and degree of anaemia (based on eye colour) was strongly positive in wormy season. The colour of ocular mucous membrane was found to have negative correlation with the PCV and Hb values.



**Comparison of extent of anaemia based on visual inspection of eye and actual PCV value:** Out of 243 observations, a total of 28.4% of the estimates fell outside the corresponding categories. However, 21.4% of the mistakes were not serious as PCV values of the animals were not low enough (> 22%) to merit drenching and in these anaemia was less severe than estimated compared to 7.00% in which the anaemia was more severe and rated as normal based on colour of ocular mucous membrane. It is common knowledge that during the course of fetal haemonchosis, the colour of the conjunctiva of sheep changes from red of healthy sheep through shades of pink to practically white as a result of a progressively worsening anaemia. Thus, in order to bridge the gap between ability to identify anaemic animals more accurately on visual inspection and to obtain similarity among results with different observers, a coloured chart with illustration was produced to assist on-farm evaluations. The chart show the intensity of colour for conjunctival mucosae of sheep with PCV of >32% (category 1), 28-32% (category 2), 23-27% (category 3), 18-22% (category 4) and <18% (category 5).

**Status of anaemia on the basis of conjunctiva colour in adult sheep during 2008-11:** The average monthly eye colour score for flock (on 5 point scale) during the period of 3 years ranged between 2.07 (March, 11) and 3.01 (September, 08). The results of screening on the basis of colour chart exhibited that monthly proportion of sheep falling in category 4 or 5 (anaemic) varied from nil (January to March, 11) to 17.35% (September, 08).

**Magnitude of FEC in visually anaemic adult sheep:** The mean FEC for animals categorized as anaemic on the basis of colour chart (category 4-5) ranged from nil (December, 10) to 13681.0±2704.9 (October, 10). On comparison of intensity of strongyle infection between whole flock and visually anaemic flock it has been observed that magnitude of FEC remained significantly higher in anaemic group during wormy season (July to November) while in other months it remained almost comparable.

*Haematological profile of visually anaemic adult sheep:* The mean PCV values in anaemic animals ranged from 14.48±1.12% (October, 10) to 24.75±1.96% (July, 10). Similarly mean Hb concentration varied from 5.24±0.26 (October, 10) to 8.75±0.25 g% (March, 09). Further it was observed that anthelmintic treatment along with better nutritional care through reduced stocking pressure of anaemic animals able to restore the haematological values.

*Observations on peri-parturient rise in FEC in sheep under TST approach:* The magnitude of FEC revealed that there was absence of peri-parturient rise in FEC in sheep flocks of arid region. The mean FEC in anaemic group ranged from 660.0±448.2 (dry stage) to 125.0±75.0 (3-7 week of lactation) compared to 334.6±99.4 (dry stage) and 218.8±53.4 (3-7 week of lactation) in visually normal ewes. The PCV and Hb values in anaemic group remained within normal limit. The study suggested that there is no need of anthelmintic treatment in lactating ewes even if they appear anaemic.

*Impact of TST on flock performance:* The estimated comparative performance of TST and en-mass targeted treatment exhibited that over the period of 3 years, the frequency of anthelmintic treatment under TST was 29.31% in 2008-09 which reduced to 23.99% in 2009-10 and further to 15.50% in 2010-11 with an average of 22.93±4.03% per annum. During the period of 3 years no adverse effect of TST were observed on flock performance. The cost of anthelmintic intervention in flock was only 10% of that incurred with targeted treatment particularly due to reduced use of anthelmintics.

### Forecasting system for gastrointestinal nematodes

*Bioclimatographs:* These explain the distribution in space and time of pasture nematodes and represent the first rational attempt to utilize climatic observations to explain important features of epidemiology of helminthic diseases. Based on epidemiological studies, the genera considered to be of importance for Rajasthan were *Haemonchus*, *Trichostrongylus* and *Oesophagostomum*. It was observed that for *H. contortus*, bioclimatographs prepared by plotting  $T_{max}$  and TRF gave better prediction in all the zones whereas for *Trichostrongylus*, precise prediction was obtained with bioclimatographs prepared by incorporating  $T_{min}$  and RH.

*Mathematical modeling for forecasting of *H. contortus* in sheep in Rajasthan:* It was attempted to obtain more precise prediction of pattern of GIN (particularly *H. contortus*) in sheep. An out line of mathematical model based on development, mortality and establishment rate, climatic data, translation of larvae on pasture, fecundity of parasite, stocking density and migratory behaviour of larvae was used to predict the level of pasture contamination and subsequent possible intensity of infection in sheep. Monthly total rainfall, average maximum and minimum temperature and relative humidity were used to determine the period in which worm eggs from faeces reach the infective larval stage and migrate on to the herbage. The steps involved were (i) interaction between FEC and climate, (ii) development rate from eggs to  $L_3$ , (iii) larval survival rate, (iv) larval migration (vertical) rate on grass blade, (v) stocking density on community grazing land, (vi) seasonal herbage availability, (vii) dry matter consumption rate, (viii) faecal output rate, (ix) establishment rate of adult worms in sheep and (x) fecundity rate of adult worm:

All the steps enlisted above were arranged sequentially and organized in “visual basic” programme to develop forecasting system for GIN in sheep of Rajasthan (**FROGIN**). It is a computer based mathematical modeling of *H. contortus* population tailored for different zones of Rajasthan. It give result as predicted FEC on start of month, intensity of FEC for next 60 days and pasture larval burden for that month. Studies on testing and validation of FROGIN in farm and field flocks of both agroclimatic conditions of Rajasthan exhibited that while considering a variation of 500 epg in FEC as non-significant, the FROGIN based forecast about intensity of infection was > 80% in agreement in all the location and management system except in arid farm where agreement % was 66.7. The low agreement in arid farm could be due to creation of artificial environment suitable for worm propagation by practicing the irrigation means to cultivate the fodder. Variation in predicted and observed FEC to the tune of > 500 was noticed

mainly during monsoon season and this could be due to variation in quantity of herbage and dilution of infection on pasture.

### Alternative control methods for gastrointestinal Nematodes

#### Genetic resistance to nematode parasites in sheep

Malpura and Russian Merino crossbred found to be tolerant to helminthic infection whereas Rambouillet and RXM crossbred sustained heavy losses due to GIN. The exotic breed and higher crosses are more susceptible to worm infection than the half breeds and natives. It was found that there was no significant effect on epg due to variation in energy level under stall feeding conditions.

*Studies on genetic variation in sheep for GI nematode infection:* The study was initiated in 1996-97 taking 4 breeds (Awassi x Malpura, Malpura, Avikalin and B. Merino). The initial result revealed that in Malpura breed out of 74 progenies of 13 sires tested, 8 were under low FEC group. In Avikalin out of 11 sires, 8 sires were under moderate level of infection whereas in B. Merino, majority of sires was in high FEC group.

*Comparison of response to repeated challenge of *H. contortus* in sheep:* The study was undertaken to evaluate the host response to repeated challenge of *H. contortus* in Avivastra sheep. It was found that FEC, Hb, PCV, MCHC and body weight were reasonably repeatable with significant repeatability around or above 0.5. The correlation among various parameters showed that log transformed worm count had significantly positive correlation ( $r= 0.836$ ) with transformed FEC and significantly negative correlation with Hb ( $r= - 0.678$ ), PCV ( $r= -0.667$ ) and TEC ( $r= -.587$ ). Similarly transformed FEC had negative correlation with Hb, PCV, TEC and body weight. Multiple regression analysis revealed that both the transformed FEC and PCV together accounted for 73% of the total variation in the transformed worm burden. Hb accounted for an additional 4% of the variation while transformed FEC, Hb, PCV, TEC and body weight factor together accounted for 86% of the observed variation in transformed worm burden. It was concluded that FEC and PCV was reasonably reliable phenotypic marker for assessing resistance to GIN in sheep.

*Heritability estimates of FEC in sheep:* In the initial phase, based on pre-treatment FEC of 300 progenies from 25 sires (pooled over 3 years) of Avikalin breed, the heritability estimate ( $h^2$ ) of FEC was calculated to be  $0.35 \pm 0.18$ , which was suggestive of existence of within breed variation and feasibility of selection for worm resistance. Further the study was undertaken at large scale since 2004 and by using FEC as phenotypic marker for within breed selection of resistant and / or susceptible animals at CSWRI, Avikanagar it was observed that this trait is low to moderately heritable. The FEC data for all the progenies born during 2004 to 2010 were pooled for estimation of heritability and sire variation in FEC was taken as random effect while sex and year effects were taken as fixed effect. The overall  $h^2$  estimates for log transformed FEC in naïve animals were 0.104 and 0.141 for Malpura and Avikalin, respectively. In exposed animals the  $h^2$  estimates for log transformed FEC were 0.081 and 0.043 for Malpura and Avikalin, respectively. Creation of divergent lines either resistant (R-line) or susceptible (S-line) could be possible through regular screening for FEC at naïve and exposed stage of infection and selection of progenies.

*Performance of selected lines:* No significant variation was observed in monthly body weights, annual GFY and reproductive performance of adult sheep belonging to R and S-line in both the breeds. The average annual mortality % ranged from  $6.33 \pm 0.89$  (R-line) to  $7.03 \pm 2.05$  (S-line) and average %culling / sale varied from  $6.12 \pm 2.36$  (R-line) to  $7.72 \pm 2.78$  (S-line).

*Intensity of GIN in selected lines:* It was observed that animals of R-line had lower FEC over the years compared to their counterparts in S-line. The animals of S-line required strategic as well as tactical anthelmintic intervention while animals of R-line were maintained without any anthelmintic intervention.

**Observations for animals born from selected animals:** In both the breeds it has been observed that progenies having inheritance of R-line possess comparatively lower FEC than those having inheritance of S-line. The ADG (0-12 month) and annual GFY remained slightly higher in progenies born from S-sire compared to those from R-sire.

**Polymorphism of Ovar-DRB1 gene in selected lines:** Ovar-DRB1 was highly polymorphic in both the lines R and S lines in Malpura and Avikalin. Although no association could be traced out between the frequencies of RE pattern and the lines in either of the breeds and study on more number of samples is needed to reach some conclusions.

**Bioactive forages and role of condensed tannins (CTs):** Beneficial effect on the ability of ruminants to withstand internal parasitic infection has been found with tannin-containing plants. The study conducted at CSWRI, Avikanagar revealed significant reduction in larval development rate in faeces from CT fed animals. CTs could bind to the protein available in the nutrient media for larvae leading to larval starvation and death as well as developing larvae can ingest CT, which bind to the intestinal mucosa and cause autolysis. CTs may also inactivate hatching enzymes in worm eggs (causing reduction in egg hatching) and can paralyze the body musculature (pharyngeal muscles) of larvae (leading to reduced viability of larvae). Hence, sufficient evidences are available that suggests that CTs can disrupt the life cycle of nematodes and help in worm management. On *in vivo* study, around 40-60% reduction in FEC with marginal rise in haemoglobin and packed cell volume were observed in sheep (infected with GIN) fed CTs (@ 5 % of DM) either as leaves of Khejri (*Prosopis. cineraria*) or as extract (acetone: water) of leaves compared to those offered diet having no CT.

**Phyto-anthelmintics:** Under NATP a large number of crude extracts from different plants were evaluated for antiparasitic activities and it has been found that *Malia azedarach* (Bakain), *Butea frondosa* (Palas), *Artemesia martima* (Kirmala), *Mallotus philippinensis* (Kamala), *Azadirachta indica* (Neem), *Embelia ribes* (Baberang), *Tagetes patula* (Gaatakadi), *Andrographis peniculata* (Kalmegh), *Vernonia anthelmintica* (Kaljiri), *Vitex negundo* (Bana), *Xanthium strumarium* (Chotta Gokhru) etc possess low to high level of antiparasitic activity against different stages of GI nematodes.

**Chemical control of nematode larvae on pasture:** In the life cycle of parasite pasture plays a major role by allowing not only hatching of eggs to larvae but also acts as a source of infection for animals by providing conducive microclimate for survival of infective larvae. Thus, by targeting the infective larvae or pre-parasitic stages of *H. contortus*, could it be possible to control or minimize the level of infection with in livestock. The effect of urea on different stages of parasites as well as on controlled pasture plots was studied.

**Egg Hatching:** It was observed that exogenous nitrogen caused significant embryonic death in *H. contortus* eggs and prevented egg hatching. The hatching was reduced drastically at concentration of 1.00 g N<sub>2</sub>% (59.94±5.14%) compared to control group (90.27±1.45%). The ED<sub>50</sub> of urea N<sub>2</sub> in preventing egg hatch was calculated to be 1.1477±0.0097 g N<sub>2</sub>%.

**Larval Mortality:** *In vitro* studies on effect of urea N<sub>2</sub> on infective larvae (L<sub>3</sub>) of *H. contortus* showed around 50% death in L<sub>3</sub> with in 4 hr post-incubation at concentration of 5.00 to 7.50 g N<sub>2</sub>%. The ED<sub>50</sub> calculated was 5.9012±0.4870 g N<sub>2</sub>%.

**Coproculture:** Incorporation of exogenous nitrogen into faecal culture revealed significant difference among various level of treatment in larval recovery. The mean % L<sub>3</sub> recovery in urea treated cultures varied from 62.02±6.65 (@ 0.019 g N<sub>2</sub>%) to nil (@ > 5.00 g N<sub>2</sub>%). It was observed that increase in total faecal N<sub>2</sub> by 3.7% resulted in only 39.2% L<sub>3</sub> recovery compared to 100 % in normal faeces. Further, if the total faecal N<sub>2</sub> concentration was increased by 23.4% the larval recovery was only 0.69±0.30%.

**Translation of larvae on pasture:** In urea treated pastures the larval translation and recovery was significantly reduced by 80% when the urea application was made @ 1.25 g N<sub>2</sub>% corresponding to 27.0 kg urea / ha.

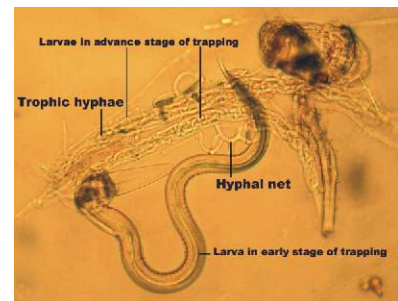
**Rotational grazing:** In Rajasthan, the grazing system of farmers is automatically of rotational in nature depending upon the availability of fodder (although not perfectly systematized). Farmers maintained their animals on community grazing land / forest with the onset of monsoon where animals are drenched during mid-monsoon and continued on the same up to October. After harvesting of kharif crop the flocks are moved on such field to utilize crop stubbles for a period of one month. From November onward the flocks were again moved to community grazing land, forest or maintained on top feed with intermittent grazing on crop stubbles after rabi harvesting.

**Pasture resting:** The study conducted in semi-arid Rajasthan (using tracer lambs) revealed that sheep grazed during monsoon on spring contaminated-summer ungrazed pasture had very low FEC and worm count compared to those placed on continuously grazed contaminated pasture. The pasture larval burden was higher in general pasture than summer ungrazed pasture. Thus, resting the contaminated pasture during summer found to effectively control the pasture larval burden.

**Biological control:** Biological control agents rarely eliminate the target organism, but reduce the numbers to acceptable levels and maintain a balance between the pathogen and the antagonist. In Rajasthan, nematophagous fungi were surveyed and *Duddingtonia flagrans* is found as most suitable candidate followed by *Arthrobotrys oligospora* and *Verticillium* spp. It has been observed that prevalence rate of nematophagous fungi in sheep faeces was higher in arid region (~ 10.0%) compared to ~ 3-5% in semi-arid region of Rajasthan. Seasonal dynamics of nematophagous fungi in sheep faeces exhibited strong seasonality during the period from January to March.

On agar medium both *A. oligospora* and *D. flagrans* effectively trap the baited infective *H. contortus* larvae. Both fungi develop loops connected to form three-dimensional networks. It has been observed that *D. flagrans* possesses relatively higher trapping ability as compared to the local isolate of *A. oligospora* on agar medium. Faecal culture assays conducted to determine the effect of fungal spores on the development of infective *H. contortus* larvae in sheep faeces showed that at a concentration of 2000 conidia/ chlamydo spores per gram faeces, more than 90% of the larvae were eliminated after 10 days incubation. *D. flagrans* has a chlamydo spore which is sufficiently robust that it can survive passage through the ruminant gastrointestinal tract and pass safely through the gut to the faeces. *D. flagrans* is found superior as compared to *A. oligospora* in surviving the gut passage in ruminants. This is due to the production of abundant chlamydo spores by the fungus.

**In vivo efficacy of nematophagous fungi:** Straw based complete feed blocks (CFB) containing *D. flagrans* chlamydo spores were developed and tested at CSWRI, Avikanagar with encouraging results. Feeding trial where sheep were fed fresh fungal blocks daily for 5 days, resulted in a significant reduction in larval numbers recovered from faecal cultures, concurrent with observed growth of *D. flagrans* in almost all the agar plates during the period in which the fungal blocks were offered. The percent development of infective larvae remained low for 2 days after the fungal blocks were withdrawn and then returned to almost pre-fungal block levels. Trials conducted for estimating shelf-life of chlamydo spores incorporated into CFB showed that feeding of CFB stored for 5 weeks at room temperature produced almost similar results to that with feeding normal fungal blocks. Although feeding CFB stored for 10 weeks led to significant reduction in larval recovery and presence of fungus in faecal cultures during the offer period, the reduction was not as pronounced as with fresh and stored CFB. This reflected that viability of spores declined somewhat after 10-week storage of fungal blocks. The possible reason might be the germination of spores by moisture present in the blocks, which resulted into the reduction in the gut survival ability of the fungus. Thus the shelf life of chlamydo spores incorporated in these blocks was more than five weeks. Grazing experiment was also conducted to observe the effect of fungus on the pasture larval burden. The larvae could not be recovered from both the plots



even after one week of grazing. However, recovery of larvae started from the 2<sup>nd</sup> week of the experiment and from then on larval counts increased markedly on the plot grazed by the control group. On the other hand low levels were maintained in the plot grazed by fungus treated group.

**Nutritional Management:** The pathogenesis of *H. contortus* infection was studied in growing Malpura male lambs under stall feeding. A trickle infection with 200 larvae of *H. contortus* was given three times a week for 8 week period in infected group. The lambs were slaughtered after 10 weeks. On the basis of PCV, Hb, serum protein, albumin and body weight values, it was found that the pathophysiological effects caused by *H. contortus* infection were mild. Furthermore, no clinical signs such as mucous paleness and sub-mandibular oedema were observed. Therefore, it is likely that either the larvae caused relatively mild infection and/or high protein diet (17% CP) withheld the effects of infection.

Role of protein supplementation to lambs with clinical *H. contortus* infection primarily on the resilience and secondly on their ability to resist the establishment of the infection was studied under stall feeding protocol. The infection protocol followed caused pathophysiological effects in Chokla lambs as revealed by haematological and biochemical parameters and body weight values. The course of the disease was most severe in lambs receiving low protein (LP) diet as clinical signs of typical haemonchosis, such as mucous paleness, submandibular oedema and inappetance were clearly seen. The infection was characterized by the development of a severe macrocytic anaemia caused by massive red cell loss into the gastrointestinal tract, hypoproteinaemia, hypoalbuminaemia and reduction in serum iron concentration. Weight gain was reduced in infected lambs relative to their controls. Faecal egg counts, worm burden, per cent establishment of larvae and fecundity of females were not significantly different between groups of infected lambs, suggesting no effect of diet on parasite establishment. Both high protein (HP) and moderate protein (MP) diets resulted in higher increase in body weight as compared to LP diet.

Feed (dry matter, DM) intake was lower in low protein fed groups without any significant effect of *Haemonchus* infection. Correspondingly, intake of other nutrients (OM, CP, NDF, ADF, cellulose, hemicellulose) was lower in the LP fed groups. No significant difference in digestibility of all nutrients was observed between control and infected lambs fed with HP and MP diets, however, the digestibility of DM, OM, NDF, ADF, cellulose and CP was significantly low in infected lambs fed with LP diet as compared to control lambs. Nitrogen balance parameters showed lower value in LP and the combined effect with infection was more pronounced ( $2.30 \pm 0.64$  in LP infected vs  $4.40 \pm 0.41$  in LP control). The performance of animals on LP diet was significantly lower as evidenced from lower average daily gain, which was depressed further when combined with *Haemonchus* infection (13.4 g in LP infected vs 39.9 g in LP control). The normal animals in all the three dietary groups gained higher live weight compared to their *H. contortus* infected counterparts. In conclusion there was significant adverse effect of infection in Chokla lambs on low protein diet and the supplemental effect of protein supported better performance through higher feed and nutrient intake, increased digestibility, N balance and growth, but with a lower magnitude compared to control counterparts. Further the role of supplementation of protein on resilience and resistance of weaner lambs of different genotypes against GIN under stall feeding is being investigated.

**Assessment of economic losses due to gastrointestinal parasitism:** The estimations were made for production losses as well as for financial impact evaluation of different treatment schemes in sheep flocks by the method of partial farm budgeting. The estimated losses in sheep flocks of Rajasthan were Rs. 973.715 million / annum in adult sheep and Rs. 217.993 million / annum in yearling sheep. The cost-benefit analysis for strategic control of GIN (single drench schedule) resulted in prevention of losses to the tune of 45.53% in female and 59.00% in male sheep. The short term studies with limited numbers of animals did not give consistent results on economic losses. The economic evaluation of various schemes revealed better economic impact of TST followed by targeted approach compared to conventional drench schedule.



**Observations on the effect of *FecB* gene inheritance on FEC in sheep:** Comparatively lower incidence was observed in Garole (~ 98% *FecB* inheritance) followed by Garole x Malpura (~ 55% *FecB* inheritance) and maximum in Malpura (nil *FecB* inheritance) breed. The monthly incidence of strongyle worms ranging from 33.3% (February) to 86.0% (September) in Garole, from 40.0% (January) to 94.8% (June) in Garole x Malpura and from 59.8% (January) to 94.8% (September) in Malpura sheep. The mean monthly FECs for strongyle infection varied significantly from 70.0±17.9 (May) to 1293.0±330.4 eggs per gram (epg) (July) in Garole, from 86.7±17.6 (February) to 1203.2±174.5 epg (August) in Garole x Malpura and from 178.6±18.3 to 1868.7±211.3 epg (August) in Malpura sheep.

Interaction between *FecB* status and intensity of strongyle infection exhibited that mean FECs remained relatively at the lowest in *FecB* homozygous carrier sheep followed by *FecB* heterozygous and maximum in *FecB* homozygous non-carrier sheep. In *FecB* carrier sheep there was single peak of lower magnitude during August compared to two peaks of higher magnitude during August and October in *FecB* non-carrier sheep. The study concluded that Garole inheritance provides resistance to GI nematodes infection to some extent however, presence of *FecB* gene in Garole was not associated with intensity of infection.

The study on polymorphism of Ovar-DRB1 gene in sheep breeds having varying level of Garole inheritance revealed that with *SacI* restriction enzyme there was significant reduction in the frequency of A allele with the reduction in the inheritance of Garole. With restriction enzyme *Hin1I*, the frequency of 'B' allele was reduced with the reduction in the inheritance of Garole. With *SacII*, Garole had significantly higher frequency of A allele than its crosses and Malpura. Though *NciI* and *BstNI* also produced similar genotypes but there was no difference in genotypic frequency among different breeds.

**Studies on impact of drought on epidemiology of gastrointestinal parasitism:** The study period of 7 years (April, 04 to March, 11) were categorized in to three groups depending on amount of annual rainfall as i) above normal TRF ii) normal TRF and iii) below normal TRF. In semi-arid Rajasthan, the magnitude of monthly incidence of strongyle worm during different categories of annual TRF revealed that there was no evident effect of annual TRF on incidence of strongyle worms during the period from April to June. However, significantly higher incidence rate was observed from August to November in the years when annual TRF was below normal. In arid region, no appreciable effect of annual TRF was noticed on incidence of strongyle worms. In semi-arid region, the magnitude of monthly incidence of *Trichuris* spp remained almost similar in all three categories of annual TRF. On the other hand monthly incidence of *S. papillosus* was relatively higher during occurrence of normal annual TRF. In arid region, incidence of *Trichuris* spp was slightly higher during the years with low TRF and no major variation was observed in incidence of *S. papillosus*. Monthly incidence of *Moneizia* spp remained relatively higher during the period of normal or below annual total rainfall in semi-arid region. In arid region incidence of *Moneizia* remained marginally higher during period of normal annual TRF and lowest during the period of low annual TRF. The magnitude of incidence of *Eimeria* spp remained higher during the period of normal TRF in semi-arid region and during period of low TRF in arid region. In semi-arid Rajasthan, the annual incidence of *Amphistomes* spp found to be inversely related with amount of total annual rainfall. However, the length of infection found to be prolonged during the occurrence of TRF above the normal. The incidence of *Fasciola gigantica* and *Schistosoma* spp infection remained quit low and no definitive pattern was observed. In semi-arid Rajasthan, the monthly intensity of strongyle infection found to be not affected by categories of annual TRF, however it remained marginally lower during periods of rainfall higher than normal. In arid region there was extension of duration with relatively higher intensity of infection in the years with low annual TRF.

#### Miscellaneous studies on parasites

**Incidence of *Sarcocystis tenella* infection in sheep:** A total of 349 muscle samples of tongue, heart, oesophagus and diaphragm from sheep necropsied at CSWRI, Avikanagar were examined for *Sarcocystis* infection. The overall

prevalence rate was 37.3%. The season, organ and age of animal had significant effect on prevalence rate. The infection was highest (46.2%) in monsoon. Heart muscles had highest prevalence (56.2%). There was positive correlation between the age of animal and prevalence rate of infection. The sex and breed had non-significant effect. The species prevalent was *S. tenella* with mean cyst size of  $270.1 \pm 12.4 \times 66.3 \pm 3.1 \mu\text{m}$ .

**Efficacy trials:** Closantel was found to have 100% efficacy against both BZ-resistant and susceptible strains of *H. contortus*. However, it was observed that following treatment with closantel, *H. contortus* which constituted 38% of the larvae in pre-treatment faecal cultures reduced to nil. While, *Oesophagostomum* larval concentration increased from 62% (pre-treatment) to 96% (post-treatment). The finding has significant bearing on future epidemiology of GIN as closantel use may alter the parasite composition leading to predominance of non-haematophagus parasites in population. Closantel was also found effective against *Sarcoptes scabiei* causing 95.3 to 99.1% reduction in mite population / cm<sup>2</sup> of skin scrapping.

Ivermectin was found to be 100 % effective against GIN of sheep. The FEC in treated animals come down to nil on day 4 post-treatment and persisted upto day 30 post treatment. On day 21 post-treatment, slaughter of treated animals revealed absence of worms while 7750 *H. contortus* were recovered from untreated animals. Further, 1% solution of Ivomec was tested for its efficacy against natural sarcoptes mange in sheep. Among sheep 74.5% cases were recovered after first injection while 25.5% required second dose after 21 days. In rabbits infected with *Psoroptes* mites, injection of Ivomec (@ 0.02 ml/Kg) resulted in absence of mites in scrapping by 21 days of first injection. No reoccurrence was seen upto 6 months after treatment.

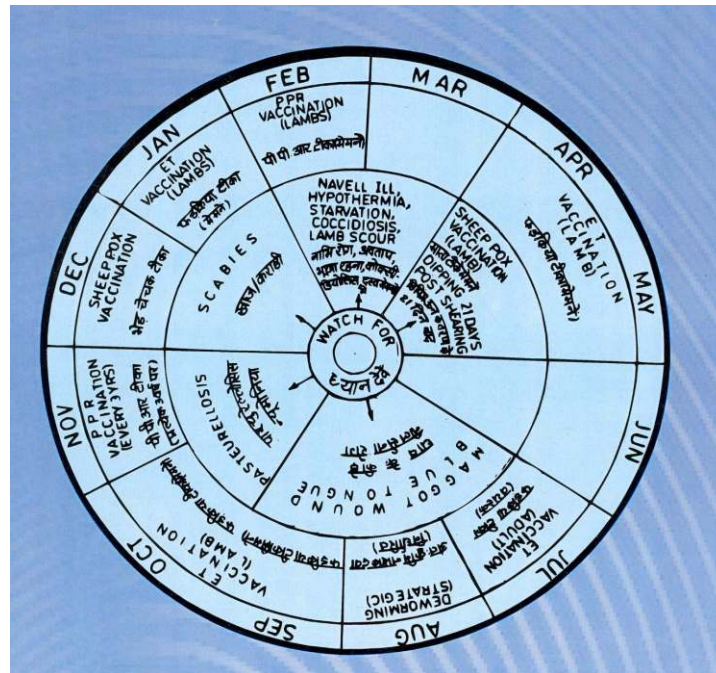
Moxidectin (@ 200 µg/kg BW s/c on 0 and 10 days) was evaluated against *Sarcoptes scabiei* at SBF, Fatehpur and on day 30<sup>th</sup> post treatment 93.3% animals showed complete recovery of lesions with absence of mites in skin scrapping.

**Bionomics of *Oestrus ovis* larvae:** The study revealed highest prevalence in June, the pupation period was 16-28 days and life span of adult fly was 8-16 days in female and 3-8 days in male. Rafoxanide was found to be 100% effective against nasal myiasis. By a strategic approach of using Rafoxanide on the whole flock during pre-monsoon period when the nasal flies emerges to lay the bots, the disease has been reduced considerably.

**Ear mange of rabbit:** Studies were conducted to observe the off host survival and infectivity of *Psoroptes cuniculi* mites in rabbits. The study revealed the survival of mites from 2 to 26 days at temperature between 2-4 and 40 °C. At 8 C and above 40 °C maintained for 24 hours 100% mortality was observed in mites. The time required for 50% mortality in mites (LT<sub>50</sub>) ranged from 13.29 to 0.89 days for the temperature intervals between 2-4 °C and 40 °C. The infectivity trials revealed that mites starved up to 7 days at 2-4 °C successfully produced viable infection in rabbits but they failed to produce clinical infection in rabbits when starved for 10 days or more at this temperature. On the basis of these results it is recommended that when time permits and where disinfecting is not practical, enclosures suspected of mite contamination be vacated for atleast 10 days before occupancy by clean rabbits.

### Flock health and preventive medicine

The division, keeping pace with recent trends of system approach in animal health management, has developed a planned flock health programme consisting of new concepts in preventive medicine, flock health and epidemiology. The flock health programme was modified time to time based on diseases pattern in flocks and as per need. The division has developed a computer based disease data information system (DDIS) for organized sheep and goat farms. This system provides facility of disease data collection, storage, processing and retrieval for future analysis.



**Morbidity profile in sheep:** It revealed that in general, non specific ailments of skin and subcutaneous tissue were the highest which included mainly wound, lameness, sprain and fracture as major constituents. These were followed by condition of general systemic state (debility, pyrexia, etc), alimentary system (enteritis etc) and respiratory system (pneumonia). The specific diseases contributed to morbidity were facial mange (*Sarcoptes scabiei*), pasteurellosis, sheep pox and blue tongue. The highest morbidity rate was in hoggets followed by suckling and lowest in weaners. The diseases of skin and musculoskeletal system were recorded maximum in all age groups followed by diseases of GSS in adult and hogget and diseases of alimentary system in weaner and suckling. Sheep pox was observed only in adult flock while BT and facial mange encountered in animals >3 month of age and pasteurellosis in animals >6 month of age. There was variation in the incidence of clinical diseases between different years. The incidence of diseases of skin and musculoskeletal system, alimentary system, GIN and BT was highest in the monsoon season while diseases of GSS and respiratory system had high incidence during summer season.

**Mortality profile in sheep:** Considering all the types of genotypes (exotic, crossbred and native) the annual EADR per 1000 animals at risk in sheep flock varied from 0.176 (6.42%) in 1989-90 to 0.817 (29.82%) in 1997 with an average of 0.360 (13.40%). The mortality in flock was high upto 1981 but following implementation of planned flock health programme it was considerably reduced except in few years. The high EADR was attributed to outbreak of sheep pox (1992-93), pasteurellosis (1994-95) and due to sudden change in feeding schedule of flocks leading to non specific debility (1996-97). In general, the maximum EADR was due to affections of respiratory system (pneumonia, suppurative pneumonia) followed by diseases of GSS (debility, toxemia, septicaemia, etc) and alimentary system (enteritis). Among specific diseases neonatal inanition, GIN, pasteurellosis, pregnancy toxemia, enterotoxaemia and sheep pox were identified as a major cause of death. Age-wise maximum EADR was observed in suckling followed by hoggets and lowest in adults. Diseases of respiratory system and GSS cause maximum death in all age group followed by diseases of alimentary and hepatic system in adult and suckling, alimentary and circulatory system in weaner and hoggets. Among specific conditions in adult sheep, GIN, pregnancy toxemia, ET, sheep pox and JD were major cause of mortality. In hoggets, pasteurellosis and GIN, while in suckling neonatal inanition and tetanus were major specific cause of death.

*Mortality profile in goats:* Over the years (2004-2010), the annual EADR ranged from 0.076 to 0.311 per 1000 goat days at risk. The major causes of deaths were enteritis, pneumonia, hepatitis, ruminal impaction, toxæmia/septicaemia, neonatal inanition etc. Ailments of GSS (mainly toxæmia) accounted for major deaths, followed by diseases/ conditions of alimentary system, and respiratory system. The month wise mortality pattern varied from nil to upto 5%. The age wise mortality pattern showed higher rate in sucklings as compared to weaner, hogget and adults. Generally male had higher EADR than female. Because of the strict implementation and monitoring of the prophylactic majors as per the health calendar the mortality as well as morbidity rates were within acceptable limit. Moreover, mortality rate in goat was significantly less than sheep at CSWRI farm.

*Mortality profile in rabbit:* Mortality data from 2003 to 2011 were analyzed with respect to EADR and causes of mortality in rabbits. Over the years the EADR per 1000 rabbit days at risk (excluding kits) ranged from 1.796 (2009-10) to 4.629 (2005-06). The gastroenteritis syndrome accounted for maximum mortality followed by toxæmia, pneumonia, cystitis, urolithiasis and coccidiosis. The EADR was higher in weaner and remained lower in adult. In general, male had higher EADR compared to females. Monthly mortality pattern revealed higher mortality from May to July and from November to February.

### **Pathology and clinical medicine**

Necropsy and histopathology were among the initial facilities developed in the Division. This enabled better diagnosis of various diseases and their pathology. Important among them investigated were the pneumonias, maedi, adenometosis, non-specific septicaemia and toxæmia, aflotoxicosis and entero-hepatopathy syndrome. Neoplastic conditions such as melanoma, fibroma, squamous cell carcinoma, synovial sarcoma and hepatic schistosomiasis were diagnosed through histopathological studies. Impact of Blue tongue virus on developing foeti was recorded and studied. The histopathological lesions of Orinjya poisoning were examined and found that lesions were similar to cyanide poisoning. Recently on histopathology Tyzzer's disease in rabbit and adenoma of uterus and pulmonary carcinomatosis in sheep was investigated.

In the last few years, the clinical pathology and biochemical laboratory facilities have been strengthened and this has facilitated investigation in metabolic and production diseases. The "Thin Ewe Syndrome" was studied but the results were inconclusive. This laboratory studied various haemato-biochemical levels of various body constitute during pneumonia and FMD. The haematology, protein profile and liver function tests indicated that debilitated animals are anaemic with low plasma protein level, supplementation with concentrate, copper, cobalt and iron gave adequate recovery. The retrospective studies on debility revealed that it was mainly associated with suppurative pneumonia, JD, GIN, wound complication and hepatitis.

In sheep, the major pathological conditions diagnosed were squamous cell carcinoma, maedi (Ovine progressive pneumonia). Suppurative pneumonia, pulmonary adenomatosis, Johne's disease, enterotoxaemia, pasteurellosis, pulmonary carcinomatosis, adenoma of uterus, lymphocytic and plasmacytic enteritis, pancreatic necrosis, hydronephrosis, obstructive urolithiasis, pyometritis, focal encephalitis, meningeo encephalitis, chronic granulomatous encephalitis, amyloidosis of kidneys, hepatitis, and hepatic schistosomiasis in sheep. Among weak animals one case of lymphocytic leukemia was recorded. In goat, cyanide poisoning lesions (due to orinjya feeding) like lung oedema and emphysema were recorded. The other conditions recorded were Pasturella pneumonia, eosinophilic enteritis, lymphocytic gastroenteritis, various forms of pneumonia, fibrinous pneumopericarditis, myocarditis, lymphoreticular hypoplasia of lymph nodes, PPR, fibroma of renal capsule, and goat pox.

In rabbit, haemorrhagic enteritis, suppurative pneumonia and other acute pneumonias were the main conditions diagnosed histopathologically. The specific conditions diagnosed were tyzzer's disease, intestinal

coccidiosis, oat cell pneumonia, cholangitis, serofibrinous bronchopneumonia, haemorrhagic myocarditis, mild fibrosis of kidney, chronic nephritis, haemorrhagic and ulcerative gastritis and mucoid enteritis.

*Investigation on debility:* The investigation indicated that during the period from November, 1990 to October, 1992, 1/10th of total morbidity was due to debility and 1/3<sup>rd</sup> of the total mortality was associated with debility. In case of morbidity due to debility, adults were more affected, followed by lambs with reference to sex, females were more affected. The maximum cases occurred in July. The debilitated lambs mostly died, or some recovered. Therefore, very few cases occurred in weaners and no case in hoggets. In adult females because of various stress factors like pregnancy, lambing, lactation, more cases occurred. Because of very little pasture available during summer and inclement weather, more cases occur by July. In case of mortality, debility was more associated with adults, and with respect to sex, more in females. The maximum cases occurred in July / February. The pregnancy / lactation stress during winters may be contributing towards February mortality. The haemato-biochemical studies on debile animals revealed low haemoglobin and haematocrit values. The debilitated group also exhibited lower levels of serum copper, serum zinc and serum manganese. The total serum protein level was higher in the control group. Within the groups of animals the wool production was almost same. The removals in the two groups due to culling deaths were almost similar. A retrospective study conducted on animals died due to debility (1985-94) revealed that the total debile deaths ranged from 8.93% (1989) to 31.57% (1991). The major conditions associated with debility were suppurative pneumonia, JD, GIN, sheep pox, wound complication and hepatitis. The total deaths due to debility were 19.04% during 1995-96 of these 24.5% were associated with suppurative pneumonia followed by GIN (15.48%) and JD (8.4%).

*Studies on anaemia:* Haematological parameters of anaemic sheep exhibited low TEC, Hb, PCV and erythrocytic indices compared to healthy group. Similarly, in anaemic sheep, blood glucose, total proteins, albumin and gamma globulin were lower compared to healthy animals. The differential count of WBC revealed higher count of PMN cells.

*Pyelonephritis:* A case of asymmetrical bilateral pyelonephritis in crossbred weaner male sheep was reported. The right kidney was highly enlarged with patchy congestion, ruptured surface and haemorrhage. On careful sagittal section, the renal pelvis and calyces were highly dilated with fluid containing pus. Similarly, the left kidney was enlarged and had shown almost similar lesions. On bacteriological investigation *C. pseudotuberculosis*, *C. renale* and *Proteus* spp were isolated. *C. pseudotuberculosis* was further confirmed by polymerase chain reaction (PCR), which yielded 551 bp, 382 bp and 285 bp amplified products of *Proline iminopeptidase* (PIP), NADP oxidoreductase and Putative oligopeptide /dipeptide ABC transporter genes respectively. Histopathologically, lesions were most severe in the medullary regions. The tubule lamina was dilated with densely eosinophilic (colloid) casts. Glomeruli were less affected in comparison to tubules. The interstitial tissues were infiltrated with polymorphs and plasma cells. The renal pelvis was infiltrated with mononuclear inflammatory cells. There has been no previous report of isolation of *C. pseudotuberculosis*, *C. renale* and *Proteus* spp from cases of pyelonephritis in sheep.

*Aflatoxicosis in rabbit:* An outbreak of aflatoxicosis was observed in rabbit unit during 1989-90. The rabbits were infected by consuming concentrate or dam's milk. Based on histopathology, outbreaks of aflatoxicosis were diagnosed during 2010-11.

*Urolithiasis:* From August 2002 to July 2008, a total of 109 animals of different breeds died due to urinary tract diseases, involving mainly obstructive urolithiasis, cystitis, urinary bladder rupture and nephritis. The EADR of urolithiasis / 1000 animals/day at risk for different breeds were Chokla (0.0032), Malpura (0.0126), Avikalin (0.0148), Bharat Merino (0.0243), Garole x Malpura (0.0283), Malpura x Garole x Malpura (0.0296) and Garole (0.061). The maximum occurrence of 52.29% was recorded in weaner lambs, followed by suckling (27.52%), adult (11.01%) and hoggets (9.17%). Sex-wise analysis revealed highest incidence was in male (92.7%). The monthly pattern showed there was increased incidence from March to July, followed by sporadic incidence in the following months. The

seasonal pattern showed increased incidence in summer months (50.46%). In all the cases no sire effect was observed on incidence of urolithiasis. Due to reduced ground water and rain, the grass cover in the summer months were very less and causes the lambs to eat sand along with grass, which may cause the imbalance in Ca: P ratio; in addition to this inadequate potable water predisposes the lambs to urolith formation. As the monsoon starts, the incidence of urolith problem started declining. The urine pH of clinically healthy lambs ranged between 7.9 and 8.1, clinically suspected animals ranged between 8.0 and 8.1 and in confirmed cases ranged between 8.1 and 8.2 revealing non significant variation among all the three groups. Many of the confirmed cases showed presence of protein in urine by Robert's reagent test. But no protein was present in urine of suspected and clinically healthy animals. In many of the clinically confirmed and suspected cases epithelial casts (10-12 / hpf), leukocytes (2-4 /hpf), RBC (0-4 /hpf) were observed. Blood picture exhibited non-significant variations for Hb, PCV, TEC, TLC, monocytes, eosinophils and basophils among different groups of animals and were within the normal range. However, a moderate increase in Hb, PCV, and TEC values were observed in confirmed cases of urolithiasis. A significantly higher neutrophil counts (56.64%) in confirmed cases of urolithiasis compared to healthy and suspected groups (37.75-43.84%) and lower lymphocyte counts in confirmed cases indicated neutrophilia and lymphocytopenia in animals that succumbed to death due to urolithiasis.

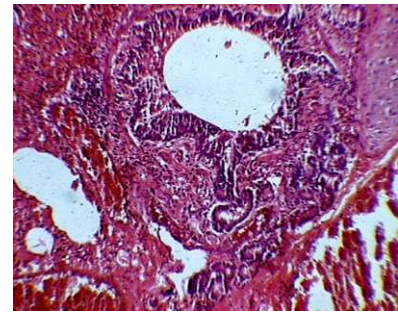


Levels of creatinine and serum urea nitrogen were found to be significantly elevated in confirmed cases of urolithiasis ( $7.05 \pm 1.42$  and  $146.34 \pm 24.87$  mg/dl, respectively) compared to healthy group. A moderate rise in levels of creatinine and serum urea nitrogen was also observed in suspected cases. The confirmed cases showed slight increase in total protein and albumin. The zinc containing enzyme alkaline phosphates was comparatively higher in confirmed cases of urolithiasis. The total cholesterol was within the normal range for the entire three groups. The mineral profile of concentrate showed imbalance of calcium, phosphorus ratio. The mineral profile of blood exhibited that there was non-significant difference in values of Ca, P and Zn among different groups. However a significant rise in concentration of copper was noticed in suspected group and magnesium was noticed in suspected and confirmed groups. The ratio of Ca: P in blood varied from 1.34:1 (confirmed group) to 1.83:1.0 (suspected group) indicating disturbed ration of Ca and P. The confirmed cases of urolithiasis animals had slightly higher phosphorus and lowered calcium in serum. The magnesium content was significantly higher in suspected and confirmed cases, but in healthy animals it was within normal range. The alkaline phosphates elevation in confirmed cases might be due to increased basal metabolic rate, excitement and pain. It is suggested that important contributing factors for the development of obstructive urolithiasis in that feedlot included rations with high levels of phosphorus, diminished water intake and dietary deficiency or lack of adequate amounts of fibre. It was found that the improper calcium-phosphorus ratio in feed, reduced water availability during summer predisposes the animals to urolithiasis. For preventive measures the chemical composition of uroliths together with environmental and dietary factors should be considered. Crystallization of urine can be prevented by dietary modification to induce urine acidification. Critical preventive measures such as providing a Ca: P ratio of 2:1 in the complete ration, increasing the salt level to 4% of the diet in order to stimulate water consumption and to increase urine volume and the maintenance of adequate and abundant water supplies should be highly considered in feedlot management.

**Pneumonia in sheep:** The pneumonic cases in sheep were diagnosed on the basis of PM lesions. On histopathological examination, out of 11 lung tissue from sheep, 5 showed pinkish exudates in alveoli, congestion, thickened interstitial space due to fibrous connective tissue proliferation and MNC infiltration and proliferation of alveolar epithelium resulting into finger-like projections into alveoli. The affected area was glandular in appearance. These lesions were characteristic to ovine pulmonary adenocarcinoma (Jaggsiekte) along with fibrinous

bronchopneumonia, bronchopneumonia, suppurative pneumonia and interstitial pneumonia. Other organs such as spleen and lymph nodes revealed fibrosis, congestion / haemorrhage. Oedematous fluid, degeneration of tubules and coagulative necrosis were observed in kidneys and degeneration and congestion of liver was observed in few cases. Fifty percent of samples from lung tissue and nasal swabs yielded 73 pure Gram positive / Gram negative bacterial isolates. The organisms presumptively identified were *E. coli*, *Proteus*, *Staphylococcus* and *Streptococcus* sp. along with non-specific isolates. With U3 gene PCR, 3 out of 13 lung samples were found positive for the retrovirus of ovine pulmonary adenomatosis.

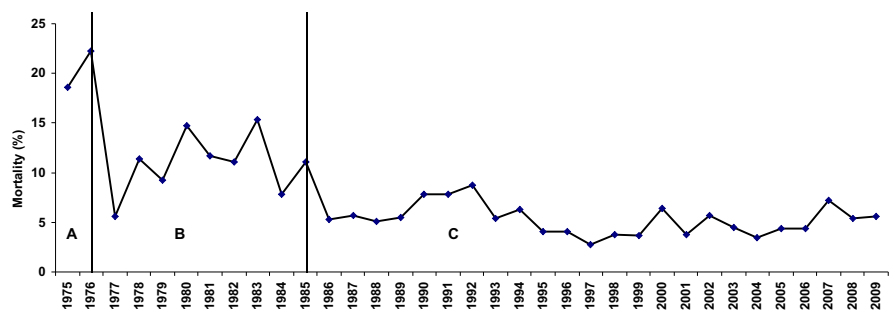
The pneumonic cases were diagnosed on the basis of clinical signs that included respiratory distress (dyspnoea, hyperpnoea, polypnoea), nasal discharge (watery, mucus tinned with thick consistency), depression, inappetance to anorexia, high rise of temperature and occasional lacrymation. Auscultation revealed occurrence of unilateral bilateral affection with doughy and moist rales. Upper respiratory tract infection (URI) was also recorded in some cases. Although, all age groups of sheep are susceptible, slightly higher prevalence was observed among nursing and weaned lambs. Adult animals having URI were successfully treated with a combination of synthetic penicillins (Ampicillin and Cloxacillin) @ 10 mg/kg body weight, 8 hly i/m and acetaminophen @ 10 mg/kg for 4-5 days along with ancilliary therapy but those revealing heavy congestion in dorso-diaphragmatic region in lung parenchyma along with moist rales did not respond favourably. One affected animal required support of bronchodilators (Etophylline and theophylline) and blood ionizer. In lambs it can not be rated well. However, lambs treated with acetaminophen @ 10 mg/kg i/m 8 hly and aminoglycosides, amikacin sulphate @ 7.5 mg/kg i/m 12 hly responded well.



**Skeletal abnormalities in sheep:** There were no histopathological changes except fibrosis in spleen, degenerative changes in liver and atrophy of glomeruli, degeneration and vacuolation of tubules in kidneys of the sheep affected with skeletal abnormalities.

**Transfer of technology**

The Division has bridged the gap in application of health care technology between organized flocks and unorganized flocks in the fields. This association has resulted in lowering of mortality in field flock to less than 5%. Epidemiologic pattern of sheep and goat pox, PPR, brucellosis and parasitic gastroenteritis in the field have been studied to suggest suitable control measures through a flock health calendar. The division offers regular diagnostic facilities to farmers and this has been found to be an effective contact point between farmers and scientists in the transfer of appropriate and need based health technologies.



Annual mortality rate in field flocks of semi-arid Rajasthan (A: Before start of programme, B: During development of programme, C: After implementation of technology)

