



# Livestock and Poultry Improvement and Management

## ANIMAL GENETIC RESOURCES

### Physical characterization and evaluation of animal genetic resources

Several indigenous breeds of livestock and poultry were physically characterized, and basic data on their production traits were generated during the year.

**Gaolao cattle:** This indigenous cattle breed is found in Amravati, Nagpur, Yeotmal and Wardh districts of Vidarbha region of Maharashtra. The Gaolao cattle is white, or grayish/black, and the muzzle and hooves are black. The average body length, height at withers and chest girth in adult females and males are 96.05, 113.99, 142.27 cm and 103.92, 128.46, 158.83 cm, respectively. Adult body weight is 219.72kg in females and 271.07kg in males. The age at first estrous (months), age at first mating (months), age at first calving (months), interval from calving to conception (days), number of services/conception, calving interval (days) were observed as 43.95, 44.83, 54.74, 150.94, 1.50, 430.26, respectively. The lactation milk yield (kg), lactation length (days), dry period (days), per day milk yield (kg), peak yield (kg) were recorded as 559.67, 249.18, 125.25, 2.43, 3.16 respectively. The highest milk yield was noticed in fifth lactation. The average fat content in milk was 4.32%.

**Bonpala sheep:** The Bonpala sheep also known as Gharpala, is distributed in all regions of Sikkim. The predominant body colour is black and white. The head is slightly convex, and ears are tubular in shape ranging from 2–8 cm. The horn is brownish-black with

- Kenkatha cattle is a valuable source of genetic material for meeting demands of future breeding programmes
- Jamunapari goats need appropriate genetic management to save the germplasm
- Ganjam and Marwari goats revealed highest genetic distance
- Indigenous poultry revealed high degree of gene diversity
- A model developed for conservation of cattle in *gaushalas*

tapering shape oriented slightly upward and backward, downward, and then twist forward and outward. The size of horn in adult male is 45.2 cm and in female is 11.69 cm. The body weight of adult male is 58.9 kg and female 51.20 kg. The chest girth, body length, height at withers measured 91.02, 81.33 and 74.6  $\pm$  0.88 cm, respectively, in adult males, and 88.46, 77.84 and 72.95 cm, in adult females. Age at first mating ranged from 1 to 1½ years. Age at first oestrus and age at first lambing were recorded as 1 year and 2 years, respectively, with a lambing interval of 1 year. The litter size is single. The number of lambing in lifetime is 6 to 7. The average daily milk yield was 313.16 g, with 9.60% SNE, 3.12% protein and 6.14% fat. Wool production was 1 kg/annum (2 shearing per annum) with average wool length 9.86 cm and diameter 54.08  $\mu$ .

**Zalawadi goat:** The Zalawadi goat, a native of the Surendranagar district in Gujarat, is a large size, sturdy and dual-

Gaolao Cattle





## LIVESTOCK AND POULTRY IMPROVEMENT AND MANAGEMENT

purpose breed. The coat colour is black. Horns are spiraled, straight and twisted (2–5 fold). Ears are long (19–25 cm), leafy, dropping with either white spot on black base or vice-versa. The nostrils are broad with slightly roman (convex) nose. Udder is symmetrical and capacious with long and outward teats. The average body weight of adult does is  $34 \pm 0.37$  kg and of bucks  $52 \pm 3.12$  kg. Zalawadi goats are high milk producers ( $1.13 \pm 0.07$  litre) with good lactation length ( $6.17 \pm 0.06$  months).

**Ghagus poultry:** Ghagus breed of indigenous poultry has its breeding tract in Kolar district, and adjoining locations of Karnataka and Andhra Pradesh. Ghagus birds are maintained largely for egg and or game purpose. The leading plumage colour is brown followed by black. The colour pattern is usually patchy in males and spotted in females. Shinning bluish black feathers exist



Ghagus poultry

on breast, tail and thighs of cocks. Neck is covered with golden feathers. Wattles are absent. Comb is red and pea or single type. Spur is short in cocks. Average adult body weight of the cock is  $2.16 \pm 0.25$  kg and hens  $1.43 \pm 0.81$  kg. Egg laying starts at the age of about 5–7 months, and hens lay about 15–20 eggs in one laying period of 20–26 days. Average annual egg yield is around 45–60. Average egg weight, albumin index, yolk index and Haugh units are  $40.25 \pm 2.39$  g, 0.07, 0.39 and  $76.79 \pm 2.93$ , respectively.

**Danki poultry:** Danki poultry is distributed in Vizinagram district and adjoining parts of Srikakulam and Visakhapatnam districts of Andhra Pradesh. The most frequent plumage colour is brown followed by black. Cocks have shining bluish black feathers on wings, breast, tail and thighs. Neck is fairly darker compared to the rest of body. Wattles are absent. Comb is red, pea type and compressed. Spur is long and sharp in cocks and small in hens. Average adult weight of cock is  $3.12 \pm 0.09$  kg and hen  $2.22 \pm 0.06$  kg. Egg laying starts at the age of about 6–8 months, and hens lay about 8–12 eggs in one laying period of around 15–20 days. Average annual egg production is only 25–35. Average egg



Dhanki poultry

weight, albumin index, yolk index and Haugh units are  $46.16 \pm 1.72$  g, 0.06,  $0.28 \pm 0.01$  and  $66.81 \pm 2.54$  respectively.

**Kalasthi poultry:** This breed of indigenous poultry is seen in Chittoor district, and adjoining regions of Nellore district of Andhra Pradesh. The common plumage colour is bluish black but brown birds are also noticed. The colour pattern is generally patchy in males and spotted in females. Cocks possess shinning golden feathers on neck and wings. Legs are proportionately longer. Wings are dorsally set revealing thighs enveloped with smooth feathers.



Kalasthi poultry

Wattles are absent. Comb is red, pea type and compressed. Spur is small in size. Average adult weight of cocks and hens are  $2.48 \pm 0.13$  kg and  $1.85 \pm 0.10$  kg. Egg laying starts at the age of 6–8 months, and yield is 9–14 eggs in one laying period of 15–25 days. Average yearly egg production is 30–40. Average egg weight is  $42.91 \pm 1.94$  g. Albumin index, yolk index and Haugh units are 0.05,  $0.35 \pm 0.02$  and  $68.81 \pm 2.19$  respectively. Kalasthi birds appear identical to those of Danki breed except that they are smaller in size, peacock type bluish in colour and have smaller spur.



### ***In-situ* conservation through Gaushalas**

Details collected from 76 Gaushalas in Haryana, Gujarat, Uttar Pradesh and Rajasthan revealed that more than half of the Gaushalas are directly or indirectly engaged in conservation of indigenous breeds. Based on the structure of cattle population in the Gaushalas, a new model, named as 10P Model, was developed for conservation of cattle in Gaushalas. Four Gaushalas have implemented the model by separating out the best cows in their herd and permitting controlled breeding by bulls of the same breed. These Gaushalas are: (i) Shri Bhadriya Gaushala—Pokhran, Rajasthan, (ii) Murli Manohar Gaushala, Bhimasar, Rajasthan, (iii) Shri Kurukshetra Gaushala—Hisar, Haryana, and (iv) Shri Gaushala Trust, Bhiwani, Haryana.

## **Genetic and molecular genetic characterization**

### ***Cattle***

**Khillar:** Genotypic data, generated across the 25 FAO prescribed microsatellite markers, was analyzed to assess the relative genetic variability. A total of 164 distinct alleles were detected across the 25 loci among the population screened. The allelic diversity per locus was 7.5. The effective number of alleles (3.8) was less than the observed values across all loci. Observed and expected heterozygosities ranged from 0.24 to 0.97 and from 0.30 to 0.89, respectively. Mean estimates of observed and expected heterozygosities were 0.58 and 0.67, respectively. High genetic diversity and absence of genetic bottleneck were observed in the Khillar breed.

**Other cattle breeds:** Comparative genetic analysis of cattle breeds, viz. Kangayam, Umblachery, Ongole, Amritmahal, Hallikar, Krishna Valley, was completed using 25 bovine-specific microsatellite markers. Gangatiri and Kenkatha cattle were also characterized. In Gangatiri cattle 138 alleles were detected with an average of  $6.273 \pm 2.394$  alleles per locus. The number of observed alleles varied from 3 to 14 with an overall mean of  $6.272 \pm 2.394$ . The observed number of alleles for all the loci exceeded the effective number of alleles, which varied from 2.007 to 6.238 with a mean of  $3.356 \pm 1.226$ . The average PIC estimate was  $0.625 \pm 0.114$ . The average observed heterozygosity was  $0.464 \pm 0.166$ , which was lesser than the expected. The average expected heterozygosity varied from 0.502 to 0.839 with mean of  $0.669 \pm 0.097$ . The f-estimates varied from  $-0.082$  to  $0.640$  with an average of  $0.318$ . Thus on an average a significant deficit (31.8%) of heterozygote exists in the Gangatiri population.

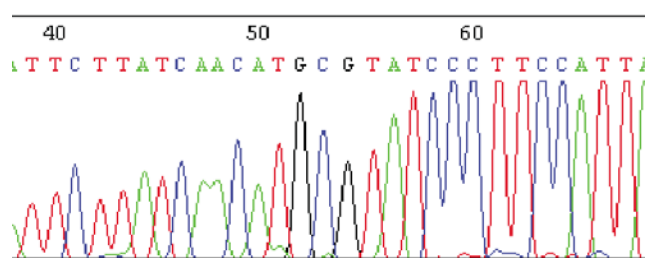
In Kenkatha cattle 125 distinct alleles were identified. The observed number of alleles varied from 2.10 to 4.75 depending on locus with a mean of  $3.41 \pm 1.09$ . The average PIC estimate was

$0.639 \pm 0.101$ . The average observed heterozygosity was  $0.540 \pm 0.171$ , whereas, average expected heterozygosity ranged from 0.530 to 0.825 with an overall mean of  $0.685 \pm 0.100$ . The f-estimates ranged between  $-0.179$  and  $0.572$  with an average of  $0.214$ . In conclusion, Kenkatha cattle still has sufficient genetic variability that can provide a valuable source of genetic material for meeting demands of future breeding programmes.

### ***Buffalo***

**Jaffarabadi:** Twenty-four heterologous bovine specific microsatellite markers, tested for buffalo genome diversity studies, were evaluated in 45 unrelated genomic DNA samples of Jaffarabadi buffaloes. Total number of alleles varied between 2 and 10 with mean of 5.04 and effective number of alleles ranged from 1.22 to 6.82 with mean 3.22. Observed heterozygosity ranged from 0.157 to 0.975 with mean of 0.554 and Nei's expected heterozygosity ranged between 0.185 and 0.853 with mean 0.604. Assessment of Jaffarabadi breed structure implied moderate extent of within breed genetic variability. Allelic frequency distribution (mode shift indicator) showed no recent bottleneck in the Jaffarabadi population.

**Expressed sequence tags (ESTs) analysis and gene annotation:** A beginning was made to establish an EST data base for Indian water buffaloes. For establishing the mammary gland specific ESTs, approximately 191,060 bases of cDNAs were generated from 466 clones of buffalo mammary gland. Summary of BLASTN



Sequence chromatogram showing mitochondrial D-loop region of buffalo

homology analysis indicated majority of clones from the two libraries (88.6%) showing significant sequence similarity to the gene sequences already registered in the NCBI database and only 11.4% sequences with no significant hits from the database. From the cumulative BLASTN and BLASTX searches a total of 402 ESTs could be assigned to the known genes in the database. The BLASTN and BLASTX analysis of buffalo mammary EST revealed that 329 ESTs from lactating and 120 ESTs from non-lactating library represented 62 and 44 unique genes/sequences respectively. Most of the ESTs that showed no homology to any known gene sequences in the database were thus classified as ESTs representing new or uncharacterized genes.





### Molecular markers for wildlife identification

Species specific molecular markers will help in identifying the species from remnants of body parts or tissues that have relevance in wild life forensic and food adulteration investigations. Mitochondrial 12 S rRNA was used as a marker for species identification and differentiation from related domestic species using PCR-RFLP approach. Cloning and characterization of mitochondrial 12S rRNA gene of chital, blackbuck, thamin deer and jackal were carried out towards this objective. The differentiation of various Indian deer species namely, chital, hog deer, musk deer, barking deer, sika deer and sambhar was done using this marker. Likewise, nilgai could be differentiated from cattle using PCR-RFLP. Methods were also standardized for forensic identification of hides of tiger and leopard and meat samples of hog deer based on morphological, chemical and/or DNA analysis.

**Polymorphism of mitochondrial DNA D-loop region of buffalo breeds:** Clustal alignment of the D-loop segment of mitochondrial DNA among Indian buffalo breeds, viz. Murrah, Jaffarabadi, Nili Ravi, Surti, Toda, Bhadawari Chilika, Mehsana, Kerala, Tarai, Nagpuri, and Pandharpuri, indicated 16 polymorphic nucleotide sites, which delineate into 20 mtDNA haplotypes. The bulk of animals grouped into majority consensus *IND- Buff* haplotype with frequency of 0.622. Frequency for other haplotypes ranged from 0.007 to 0.078 (*IND-Buff1*). Phylogenetic tree based on the mtDNA polymorphism, grouped these 20 haplotypes in different clusters close to buffaloes but distinct from *Bos* species.

### Goats

**Jamunapari:** Estimates of genetic variability measures, viz. effective number of alleles and gene diversities revealed substantial genetic variation in Jamunapari goat population in its breeding tract. Average polymorphism and expected gene diversity in the population were  $1.066 \pm 0.510$  and  $0.528 \pm 0.237$ , respectively. Population significantly differentiated into different groups, and showed fairly high level of inbreeding ( $f = 0.189 \pm 0.049$ ) and global heterozygote deficit. Bottleneck analysis indicated the introduction of unique/rare alleles by immigrants. The study revealed that Jamunapari, an improver goat breed throughout South Asia and now at risk, needs appropriate genetic management for its immediate conservation and improvement.

**Osmanabadi:** Genetic variation at 21 FAO approved microsatellite loci, population structure, and genetic bottleneck hypothesis were examined for Osmanabadi goat population from Maharashtra state. The observed and expected homozygosities ranged from 0.2609 to 0.9302 and 0.1148 to 0.8961, respectively, whereas heterozygosities ranged from 0.1087 to 0.7391 and 0.0999 to 0.9004 respectively. The Nei's heterozygosity was minimum

(0.0986) at locus ILSTS029 and maximum (0.8906) at ILSTS033. The average number of alleles was 6.85 with maximum (16) allele at ILSTS 033 and minimum (2) at ILSTS 065.

**Sangamneri:** Genetic diversity measures evaluated at 17 loci in Sangamneri goat population indicated the range of observed and expected homozygosities from 0.3478 to 0.9787 and from 0.1414 to 0.9787, respectively, and that of heterozygosities from 0.0213 to 0.6522 and 0.0213 to 0.8586 respectively. The Nei's heterozygosity was minimum (0.0211) at locus OarJMP29 and maximum (0.8495) at OarFCB304. The average number of allele was 6.70 with maximum alleles (13) at ILSTS 058 and minimum (2) at OarJMP29.

**Sirohi:** The observed and effective number of alleles at 25 microsatellite loci varied from 5 to 25 and from 2.05 to 14.07. The average observed and expected heterozygosity across the studied loci was 0.50 and 0.79 respectively. The allelic richness, observed independent of sample size, across the loci varied from 4.30 to 19.52. The population analysis based on microsatellite markers revealed substantial genetic variation in the population, which may be exploited for further improvement of the breed through appropriate breeding strategies.

**Genetic diversity of indigenous sheep populations:** Variation in the ovine intermediate filament type 1 (IF6) and high sulphur wool protein, (B2C) gene loci was investigated in Garole, Chokla, Kheri, Sonadi, Marwari, Chhotanagpuri and Jalauni sheep. Polymorphism at the IF6 loci revealed a biallelic pattern after cleavage with Msp1, whereas, Bsr1 RFLP analysis of amplified product of B2C locus exhibited three polymorphic patterns, one with 350 bp and 225 bp (XX genotype); second with 309 bp and 225 bp (YY genotype) and third with 350 bp, 309 bp and 225 bp (XY genotype). The 41 bp and 23 bp fragments were not visible on electrophoresis.

**Single nucleotide polymorphism (SNPs) in sheep and goats:** In 58 samples of Karnah sheep breed 9 distinct genotypes at GH-4 gene, viz. AA, AB, AC, AD, BB, BC, BD, CC and CD were identified with genotypic frequency of 0.447, 0.153, 0.121, 0.035, 0.121, 0.052, 0.035, 0.018 and 0.018 respectively. The alleles A, B, C and D showed frequency of 0.625, 0.222, 0.111 and 0.042 respectively. Alleles causing mutation in coding region of GH-4 were later named according to change in amino acid as per international norms; 135 M and 135 L alleles were observed and 135 L was more frequent. For GH-5 SSCP haplotypes A, B, C, D, E, F, G and H were found with 34, 20, 2, 6, 26, 4, 4 and 4% respectively.

In Black Bengal goats, GH-4 exon showed A, B, C, D, E and F haplotypes with 2.17, 10.87, 15.22, 6.50, 45.55 and 23.90%, respectively, whereas GH-5 exon showed A, B, C, D and E variants with 14.65, 77.00, 2.09, 2.09 and 4.17% respectively. Growth hormone, growth hormone receptor gene and beta lactoglobulin



gene were screened in Jamunapari and Jhakrana goats using PCR SSCP, and variants were sequenced. New DNA sequence variants were analyzed using DNASTar, and 65 NCBI accessions were obtained. Complete growth hormone gene was studied in Sangamneri goats. The sequences of 13-364, 345-693, 1011-1344, 1648-2012, 1993-2344 and 2325-2506 nucleotides were generated, and 6, 7, 9, 11, 6 and 5 SSCP variants were identified, respectively.

**Milk protein diversity in Indian goats:** Milk protein polymorphism was investigated in Ganjam, Marwari, Jamunapari, Jakhraha and Sirohi goats. In the studied population 13 different alleles were observed. The average number of alleles was lowest in Ganjam goats and highest in Sirohi goats. The effective number of alleles was observed in the range of 1.26 to 1.56, over all the breeds. The percentage of polymorphic loci varied from 50 to 100% over different genetic groups. The Shannon's weaver index over each locus showed highest diversity values for  $\alpha_s1$  and lowest for  $\beta$ -LG locus (0.01).

The highest genetic distance was observed between Ganjam and Marwari goats. Ganjam goats exhibited highest distance among all the breeds except local goats of Madhya Pradesh and Uttar Pradesh. The least genetic distance for milk protein gene was observed between Barbari-Jamunapari (0.994) and Jamunapari-Jakhraha (0.994). Four major clusters were observed in genetic tree indicating Marwari in one group, local MP in second cluster, Ganjam and local UP in third cluster, and Barbari, Beetal, Jakhraha, Sirohi, Jamunapari in fourth cluster. Jakhraha and Beetal were more similar in milk protein gene evolution than other goats. Jamunapari was more similar in milk protein evolution with Sirohi.

## Horse

**Zanskari:** In Zanskari breed of Indian horse 145 alleles were observed across all the 25 loci evaluated with a mean of  $5.8 \pm 1.32$ . The mean effective number of alleles were  $4.95 \pm 1.18$  indicating high level of genetic variability within breed. This was also reflected in mean observed and expected heterozygosity of  $0.61 \pm 0.06$  and  $0.78 \pm 0.05$ , respectively. The polymorphism information content (PIC) varied from 0.65 for NVHEQ54 to 0.86 for HMS7 showing the suitability of these markers for studying within breed diversity. The within-population inbreeding estimates ( $F_{IS}$ ) indicated moderate levels of inbreeding. The analysis of these data revealed absence of any significant heterozygotic excess both in infinite allele model (IAM) and sequential allele model (SMM), demonstrating that the Zanskari horses have not faced any recent genetic bottleneck.

**Marwari:** Seminal plasma contains many proteins which may be used as a diagnostic tool/marker to select stallions of high breeding value. The heparin and gelatin binding proteins were isolated from the Marwari stallion seminal plasma. Five heparin binding proteins (17–83 kDa) and four gelatin binding proteins (18–83 kDa) were identified. The effect of these proteins in fertilizing potential is being evaluated.

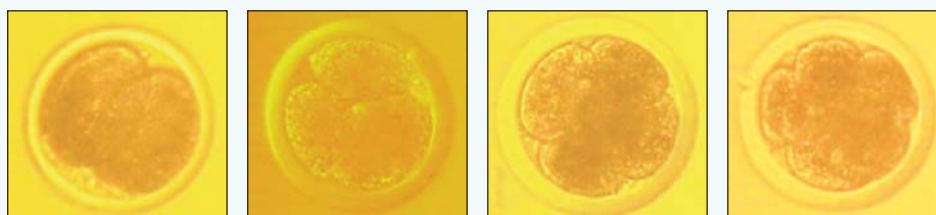
## Yak

Studies were conducted to investigate the polymorphisms in the candidate genes, viz. growth hormone (GH) gene, growth hormone receptor gene and insulin like growth factor-I (IGF-I) gene. The polymorphism in IGF-1 is associated with birth weight. The

### Production of cloned buffalo embryos through nuclear transfer (NT) of somatic cells

For the development of competent donor somatic cells, 8 cell lines BBM 16, BBM 31, BBM 32 and BBM 79 from male buffalo donors and BBF 22, BBF 33, BBF 37 and BBF 82 were cultured for 24 consecutive passages. The cell lines showed stable karyotype up to 12th passage in > 92% cells but subsequently, the 50 XX/XY normal chromosomal complement was reduced with the increase in passage number. At 24th passage, the percentage of normal cells ranged from 69.5 to 82.5% in different cell lines. The major chromosomal aberrations were

polyploidy and aneuploidy types. The cell lines showed decrease in average mitotic index of 17.85% in early passage cells to 12.3% in cells at 24th passage. The size of donor cell was also studied. The cells of 12–15 microns produced better cleavage rates in SCNT oocyte cell complexes. The oocytes showing uniform cytoplasm and organized polar body in the perivitelline space, showed better rates of enucleation and cleavage after activation of cell + oocyte complex. The NT derived 2-cell, 4-cell, 8-cell, 16-cell and 32-cell morula stage were obtained.



The NT derived morula at different cell stages



random amplified polymorphic DNA (RAPD) assay using 10 random decamer oligonucleotide primers indicated that all phenotypically different types of Indian yaks are genetically similar.

### Poultry

The Dahlem Red and White Leghorns were genetically characterized using different molecular tools. On PCR-RAPD analysis, the halfsib and non-inbred groups showed higher genetic distance (0.76). The intra population genetic similarity was higher in the inbred groups. The genetic similarity between IWH and IWI lines of White Leghorn was highest (0.8) and between IWH and IWK the lowest (0.71). Line-specific RAPD markers were identified. The cluster analysis of these four populations indicated that IWH and IWI were genetically closer to each other, while, IWK was genetically distant from the other three populations.

**Genetic diversity in indigenous poultry:** Attempt was made to define all the 15 defined chicken breeds of India using microsatellite markers. The data generated was utilised to establish population parameters and genetic relationship among them. All the loci selected exhibited high polymorphic information content and gene diversity. All the breeds/populations also revealed high degree of gene diversity.

**Disease resistance:** HSRBC (771) and LSRBC (373) chicks of IWG divergent lines after third generation of selection based on humoral immune response to sheep red blood cells (SRBC) were evaluated for layer traits, viz. BW20, BW40, EW28, EW40 and EP40. The LS means of these traits in the HSRBC lines were  $136.93 \pm 0.96$  day,  $1340.96 \pm 26.54$  g,  $1404.47 \pm 30.76$  g,  $45.52 \pm 0.29$  g,  $46.80 \pm 0.51$  g and  $88.45 \pm 1.66$ , respectively. The corresponding values in the LSRBC line were  $138.51 \pm 1.77$  day,  $1407.66 \pm 16$  g,  $1507.61 \pm 19.09$  g,  $47.33 \pm 0.59$  g,  $47.88 \pm 0.83$  g and  $91.46 \pm 1.90$ . LSRBC line demonstrated higher BW20, BW40 and EW28.

In broiler, in  $F_3$  generation, the overall mean for SRBC response ( $\log_2$  of titre) in high and low responding lines were  $9.100 \pm 0.197$  and  $5.551 \pm 0.203$ . Corresponding CMI (% thickness) were  $88.259 \pm 4.610$  and  $37.205 \pm 4.628$ . Serum lysozyme (ig/ml) and IgG (mg/ml) levels were  $7.59 \pm 0.01$  and  $3.91 \pm 0.084$  in HIC line and,  $6.23 \pm 0.076$  and  $2.54 \pm 0.071$ , in LIC line. The candidate genes, viz. BL-bII, IFN-g and IL-2 promoters were analyzed by PCR-RFLP that exhibited gene and genotype frequency differences between immunodivergent lines.

### Functional genomic studies of livestock

**Gene analysis of milk performance traits in *Bos indicus* breeds:** DNA samples of unrelated animals from 11 indigenous cattle breeds, viz. Sahiwal, Hariana, Tharparkar, Rathi, Nagori, Mewati, Kankrej, Red Kandhari, Deoni, Dangi, Gaolao, were evaluated by genotyping at two of the candidate loci, viz growth

hormone (b-GH) and kappa casein ( $\kappa$ -cas) affecting milk production/composition traits. Analysis revealed that AA genotype and A allele were most commonly distributed in Indian indigenous cattle populations, followed by AB genotype and B allele. No animal with homozygous BB genotype was observed in any of the studied population.

**Genes involved in the immune response regulation:** Nitric oxide synthetases are actively involved in the inflammatory process during the ensuing of the immune response against an infection. The promoter region of the NOS2 gene in *Bos indicus* breeds and the Holstein Friesian cattle was amplified and sequenced. An SNP was observed at nucleotide position 5,271 in the promoter region of the NOS2 gene in the *Bos indicus* cattle. The nucleotide sequence of the promoter region of the Sahiwal, Tharparkar, Hariana and Rathi cattle, showed the presence of both C and A at position 5,271 in certain animals in variance to *Bos taurus*, which had only C at this position.

### Conservation of animal genetic resources

#### In-situ conservation

**Beetal goat:** Villages (55) from Beetal breeding tract in Gurdaspur district were identified. The herd size of adult goats ranged from 6 to 32 with the goat breeders and the entire feeding system is based on intensive grazing system. Elite goats (176) with goat breeders were identified and ear marked with brass tags. Their registration was made in record. All the selected goat breeders were given the part maintenance cost for elite goats.

#### Ex situ conservation

**Krishna Valley cattle:** About 170 Krishna Valley animals including breeding bulls, bullocks, lactating cows, calves of different ages and heifers were identified from Belgum, Bagalkot and Bijapur districts and tested for diseases like brucellosis,

Krishna Valley bull







tuberculosis, John's diseases etc. In view of the endangered status of the breed only seven bull calves were brought to the Central Research Station (CRS), Uruli Kanchan, and are being monitored regularly.

**Jaffarabadi buffalo:** Jaffarabadi buffaloes are found mainly distributed in Junagadh, Amreli, Bhavnagar and Rajkot districts of Saurashtra region. Bull calves were kept for rearing at Nanodra



Jaffarabadi buffalo

farm in Gujarat State and subsequently brought to the Central Research Station, Uruli Kanchan.

**Poultry:** Multiplication of purebred Aseel (Peela), Kadakanath, Naked Neck, Frizzle fowl and CARI Red was continued and all these breeds were fully utilized for the production of commercial crosses of scavenging chicken, viz. CARI Nirbheek, CARI Shyama, Upcari and Hitcari. Aseel revealed much higher immunoresponsiveness to SRBC. Body weight of females at 20 and 40 week, age at sexual maturity, 40 week egg number and egg weight for Naked Neck line were 1,098 g, 1,485 g, 141.37 days, 97.69 eggs and 56.57 g, respectively, and the corresponding values for Frizzle line were 928 g, 1,350 g, 144.8 days, 89.19 eggs and 54.75 g. The attempts were initiated to develop the molecular standards for trueness of Red Jungle fowl. The eggs of Red Jungle fowl were collected from outside the protected areas of forest range in district Pilibhit (Uttar Pradesh). and hatched under captivity.

Adult Red Jungle fowl male and female (inset : eggs)



### Genetic manipulation through introgression of major gene, transgenesis and reconstitution of poultry:

Two Naked Neck pure broiler populations having Naked Neck gene (NNWP and NNCP) have undergone specialized selection programmes over 11 generations and showed consistently relative superiority of Naked Neck birds over normal for economic broiler traits. The realised response for primary trait (5-week body weight) was statistically significant in both lines. The heritability estimates within generation for 5-week body weight ranged from moderate to high in both the lines.

Indirect selection for general immunocompetence using high HI titre method was initiated in two Naked Neck populations along with direct selection for high 5-week body weight. The dynamics of antibody response and the estimation of genetic and phenotypic response to ND vaccine were evaluated after 5-week post vaccination. The average HI titres in male and female progeny of NNCP were  $4.09 \pm 0.07$  and  $4.03 \pm 0.06$ . Among genotypes, homozygote birds showed highest 5 week body weight as 973 g followed by heterozygote (966 g) and normal birds (877 g). The HI titre was 4.04, 4.05 and 4.11 in homozygote, heterozygote and normal birds, respectively. Total birds were further classified as low, medium and high responders on the basis of HI titre. The respective mean values estimated in these classes were 1.11 (low), 4.62 (medium) and 6.47 (high). More or less similar results were obtained in NNWP population.

### Molecular reproduction studies on bovines

- Use of simple media like mCR2aa and mSOFaa for *in vitro* culture of buffalo zygotes resulted in higher yields of day-8 morulae and blastocysts or day-9 blastocysts, even without serum supplementation and co-culture with somatic cells.
- Culture of inner cell mass cells on a homologous fetal fibroblast feeder layer was found to be a better choice than their culture on gelatin coated dishes for production of embryonic stem cells in buffaloes.
- Partial gene sequence (196 nucleotides) of buffalo ovary IGF I exhibited homology with cattle (99%), sheep (98%), goat (97%), pig (93%) and human (93%). Partial IGF II gene sequence (154 nucleotides) showed homology with cattle (100%), sheep (100%), pig (92%) and human (88%).
- Angiotensin II capacitated buffalo spermatozoa at nM concentration through AT 1 receptors on the sperm surface.
- Oviductin genes from oviductal tissues of buffalo and goat were cloned and sequenced.
- Lysozyme gene from abomasum and mammary gland tissue of buffalo was cloned and sequenced.
- Human lactoferrin cDNA from mammary gland tissue was cloned and sequenced.



## ANIMAL BREEDING

### Cattle

**Frieswal:** The synthetic breed development programme through crossbreeding continued during the current year also. The total population of Frieswal females at 43 Military Farms (MF) was 19,045 including 10,388 adult cows, 6,739 young stock and 1,918 calves. Presently, PDC has 305 doses of pure HF semen and 408,179 doses of Frieswal semen in the gene bank. The overall mean of age (AFC) and weight (WFC) at first calving in Frieswal cows was 978.8 days and 374.7 kg, respectively. The effects of farm and season and year of birth were significant on AFC and WFC. The overall least squares mean of 300 days milk yield (MY 300), total milk yield (TMY), peak yield (PY) and lactation length (LL) was 3,248.3 kg, 3,303.7 kg, 14.89 kg and 323.6 days. Estimates of heritability for all reproduction and production traits were low ( $0.003 \pm 0.01$  to  $0.10 \pm 0.01$ ).

### Indigenous Breed Project

**Hariana:** The overall conception rate in the herd was 53%. Average age at first calving, first lactation milk yield and peak yield were 1,538 days, 833 kg and 5.47 kg, respectively. First dry period, service period, lactation length and calving interval averaged 201, 147, 205 and 453 days, respectively. Young bulls were put to training for draught and took an average of  $37.22 \pm 2.1$  days to be ready for draught purposes. The mean fatigue score for empty cart ranged between 2.16 to 2.33 after 2 h work and 2.44 to 2.66 after 3 h work. In a cart with 8 q load, the corresponding values were 3.05 to 3.55 and 3.72 to 4.22, respectively. Average carting ability (as per C K Thomas method) was 13.11.

**Ongole:** The female herd strength was 1,330. The breeding population contained 834 females and 9 breeding bulls. Forty-one bulls have so far been used under test mating in 5 sets. So far 2,179 daughters have been produced through 5 sets. The semen doses available on closing date at GP Unit were 134,189.

The per cent cows in milk, wet average per day per cow and herd average per day were 36.61%, 3.308 kg and 1.16 kg, respectively. The age at first calving averaged 53.53 months. The average lactation milk yield and peak yield was 439.9 and 3.01 kg. The overall average of service period and lactation length was 203.6 and 157.3 days, respectively. The dry period and calving interval averaged 350.9 and 515.1 days.

Draught studies were undertaken on animals by using single harness plough with digital dynamometer. Rectal temperature, respiration rate/min and pulse rate/min before work varied from  $99.6^\circ$  to  $101.7^\circ\text{F}$ , 18 to 28 and 40 to 52, and after work ranged from  $100.6^\circ$  to  $103.2^\circ\text{F}$ , 32 to 58 and 52 to 64, respectively. The results revealed that draught power varied from 0.70 to 0.92 HP among the bulls.

- Lactation length of 324 days was observed in Frieswal cows
- Elite herds of selected buffalo breeds were established under Network Project on Young Bull Production
- Barbari goats showed very high reproduction efficiency
- Survey on pig husbandry status was completed in eight states of North East
- Fertility, hatchability and survivability till lay improved over the preceding generation in IWH and IWI lines of poultry birds
- CARI Sonali secured second position in RSLT at Gurgaon
- Improvement of shank length was given emphasis in selection of Vanaraja male line
- Vanaraja female line was improved for egg production

### Field progeny testing

At Ludhiana, 128 bulls have so far been used in six different batches. Overall conception rate was 41.4% on total inseminations basis and 46.2% on total AI's follow up basis since inception of the project. First lactation records were completed by 761 daughters from first four sets of bulls. The milk yield showed increasing trend among the progenies of different sets. The frozen semen of 20 bulls from sixth set and 25 crossbred bulls of seventh set was used for performing 4,430 artificial inseminations. The conception rate was 39% and during this year 1,736 pregnancies were confirmed. Female progenies from fourth batch completed their first lactation record. Their average milk yield was 2,285 kg and average age at first calving 1,200 days.

At BAIF, Pune, 104 bulls have so far been used in five batches since inception of the project. The overall conception rate was 41.2% on total inseminations basis. First lactation was completed by 1,525 daughters from first three sets of bulls. The average 305 days milk yield was observed to be 2,930.34, 2,821.75 and 3,050.94 kg, respectively, for three sets. The age at first calving indicated trend in desired direction as it decreased from 976.5 days for the progeny born from the bulls used in first set to 963 days for the progenies of fourth set. Fifth batch of bulls was used for genetic evaluation 2,461 inseminations were carried; and 733 pregnancies were confirmed. The cumulative conception rate of this batch was 44.6% (range 31.2 to 56.5%).

### Buffalo

**Buffalo improvement programme:** Four genetically superior breeding buffalo bulls and 372 frozen semen doses from progeny tested bulls were disseminated to various buffalo breeding agencies involving village Panchayats, individual breeders and State Animal Husbandry Departments at Hisar.

**Network project on buffalo improvement:** Genetically





superior Murrah breeding bulls (129) were selected from various centers of the Project for Murrah Breed, and their semen was frozen for progeny testing programme. At present 488,555 doses of frozen semen from genetically superior bulls are in stock. Elite herds of Nili-Ravi, Surti, Jaffarabadi, Bhadawari, Pandharpuri, Godavari and Swamp buffaloes were established at the respective centres of Network Project for Young Bull Production.

### Sheep

**Meat purpose:** Avikalin, Malpura, Garole  $\times$  Malpura, Chokla, Bharat Merino, Marwari, Magra and Gaddi breeds of sheep were selected for improvement. Among these the Bharat Merino, Avikalin and Garole  $\times$  Malpura have been developed at the Institute whereas the other indigenous breeds are being improved through selection. The survivability and culling in Avikalin flock was 92.70 and 4.91%, respectively. Topping per cent of the flock was 93.43%. Body weight indices for Avikalin and its crosses at birth, 3, 6, 9 and 12 months of age were 2.89, 14.15, 19.45, 20.61 and 24.30 kg, respectively. The corresponding indices for the pure Avikalin were 2.84, 13.61, 19.61, 21.00 and 24.76 kg, respectively.

Purebred stock of Malpura, the local mutton breed and, Garole, highly prolific breed are being maintained and crossed to enhance reproductive efficiency of the former. The targeted 6-month body weight in Malpura sheep was achieved. Least-squares means (LSM) of body weights at birth, 3, 6, 9 and 12 months were 3.17, 14.87, 21.55, 22.28 and 26.52 kg in Malpura; 1.86, 9.62, 13.40, 16.24 and 17.49 kg in Garole  $\times$  Malpura (GM): 3.05, 13.70, 18.65, 21.69 and 24.583 kg in GM  $\times$  Malpura; 2.24, 11.10, 15.87, 18.68, 22.04 kg Malpura  $\times$  GM, respectively. Increase in body weight of (GM) M lambs was 64, 42, 39 and 40% at birth, weaning and 6-month of age, respectively.

### Goat

**Barbari:** Genetic improvement of the Barbari goat is being carried out through selective breeding in the nucleus flock. Use of bucks selected on the basis of index value combining 9 month body weight and 90 day's milk yield of the dam indicated the improvement in both milk yield and body weight. The annual population growth in Barbari goat was 142%. The overall least squares means for body weight at birth, 3, 6, 9 and 12 months of age were  $1.89 \pm .01$ ,  $7.95 \pm 0.06$ ,  $12.51 \pm 0.11$ ,  $18.10 \pm 0.13$  and  $22.02 \pm 0.17$  kg respectively.

The overall mean for lactation milk yield, milk yield 90 days, milk yield 140 days and average daily milk yield were  $78.5 \pm 2.4$ ,  $69.0 \pm 1.9$ ,  $110.2 \pm 4.4$  and  $0.673 \pm 0.17$  liter, respectively. Lactation length averaged  $116.4 \pm 1.4$  days. The Barbari goat has very high reproduction efficiency. During the year the kidding rate was 1.62. The breeding efficiency on the basis of does available



Superior Barbari buck for breed improvement

and on the basis of does topped were 139 and 80.5% respectively. There had been improvement in overall reproduction rates of the breed over years.

**Jamunapari:** Genetic improvement of the Jamunapari goat is being carried out through selective breeding in the nucleus flock. Use of bucks selected on the basis of index value combining 12 month body weight and 90 day's milk yield of dam indicated improvement in both milk yield and body weight. Mean body weights of kids at birth, 3, 6, 9 and 12 months of age were  $3.29 \pm 0.03$ ,  $11.51 \pm 0.11$ ,  $15.08 \pm 0.18$ ,  $21.81 \pm 0.34$  and  $25.01 \pm 0.39$  kg, respectively, during the year. Average milk yield (kg) in 90 days, 140 days and total lactation yield were  $92.32 \pm 0.85$ ,  $129.96 \pm 1.30$  and  $156.78 \pm 2.94$ , respectively. Significantly longer lactation length was recorded in does which kidded during autumn. Multiple births were 37.5% and kidding rate on the basis

Superior Jamunapari buck for breed improvement





## LIVESTOCK AND POULTRY IMPROVEMENT AND MANAGEMENT

of does available was 86.7% respectively. The ranking of sires on the basis of sire indices was computed by combining 9 months body weight and 90 days milk yield of their dams. Ten top ranking bucks were selected and allocated for breeding. More than 230 goats (128 males and 102 females) were supplied to farmers, SAUs, NGOs and other research institutions for improvement and conservation of Jamunapari goats under field conditions.

### Rabbit

**Meat and fur production:** In broiler rabbits, the 12-week weights were 1.57, 1.45, 1.39, 1.47, 1.39 and 1.50 kg in New Zealand White (NZW), White Giant (WG), Gray Giant (GG), Soviet Chinchila (SC), Dutch and Black Brown (BB) breeds respectively. Litter size at birth were 4.75, 6.00, 6.76, 5.50, 3.00 and 3.33, respectively, in NZW, WG, GG, SC Dutch and BB breed.

**Angora hair production:** German Angora rabbits (breeding flock) had average pooled wool yield in first, second, third, fourth and fifth clips, respectively, 212.36, 189.83, 184.30, 184.49 and 185.74 g. The pooled wool yields of British Angora (BA), Russian Angora (RA) and A-1 crosses were 144.85, 147.47 and 135.52 in first clip, 129.42, 136.08 and 117.45 in second clip, 127.30, 136.18 and 129.75 in third clip and 128.0, 136.66 and 114 in fourth clip, respectively. The overall equivalent average death rate (EADR) in Angora rabbits was 0.43/1,000 rabbit days at risk.

### Pig

Survey on status of pig husbandry practices regarding housing, nutrition, health coverage and product utilization by farmers in all the eight states of North East was completed. Data on haemato-biochemical parameters of pigs in respect of variation in altitude were also recorded.

**AICRP on Pigs:** Genetic stability in terms of litter size at birth through *inter se* mating in 50% Hampshire (11th generation) and 75% Hampshire (10th generation) at Khanapara, Assam was recorded as 6.67 and 6.91 respectively, in the first crop and 8.69 and 8.1, respectively, in second crop i.e. from the tried sows. With LWY after *inter se* mating in 50 and 75% crossbred, the litter size at birth was recorded, respectively, as 8.75 and 7.45, at Jabalpur; 6.87 and 7.06 at Tirupati; 5.70 and 7.19 at Kattupakakam; and 5.1 and 5.2 at Mannuthy. Due to inbreeding depression, litter size at birth at IVRI, Izatnagar, was recorded as 5 in Landrace breed of pigs. Litter size at birth at Ranchi and Goa was recorded as 5.28 and 8.25, respectively, in indigenous pigs.

### Poultry

#### Poultry for egg

**High producing commercial layer:** Six strains of White Leghorn were improved under the AICRP on Poultry Breeding using selective breeding through intra-population selection for egg



Laying birds were improved for production

production up to 64 weeks of age superimposed with independent culling level for egg weight at 28 weeks of age and layer house viability. The genetic response was positive and significant in both the strains for egg production and egg weight. The age at sexual maturity decreased in IWN and remained unchanged in IWP strain.

The S-27 generation of IWH and IWI lines was evaluated at the CARI, Izatnagar. The egg production up to 64 weeks of age increased over the last generation. The genetic response to selection for 64-week egg production/generation was positive and significant in both IWH (1.105 eggs/generation) and IWI (1.06 eggs/generation) in the last two generations. Subsequently, the S-28 generation of these populations was regenerated. The fertility, hatchability and survivability till lay of both the lines increased over the preceding generation. The strain cross HI showed marked improvement in 64-week egg production.

At the PD on Poultry, Hyderabad, White Leghorn pureline populations, viz. IWH, IWI and IWK were improved for egg production and egg mass up to 64 weeks. In the S-5 generation, the egg production and egg weight at 72 weeks were 238 and 56.6 g in IWH, 244 and 57.8 g in IWI, 222 and 60.8 g in IWK, respectively. The egg mass up to 64 weeks was 10.56 kg in IWK. The egg production increased over the previous generation by 5.2 and 3.9 eggs in IWH and IWI, respectively. Six strain crosses of a diallele cross were evaluated. The egg production up to 40 weeks was higher in the crosses, IWH  $\times$  IWK (103 eggs), IWI  $\times$  IWK (105.5 eggs) and IWH  $\times$  IWI (104.9 eggs) than others. Egg weight and body weight did not differ significantly.

The main White Leghorn strains i.e. WLH and IWI completed S<sub>27</sub> generation of selection. The reproductive performance showed an increasing trend with fertility (%) ranging from 91.36 to 92.41% in the pure strains and 93.36% in HI strain cross (S<sub>28</sub>); the hatchability (%) on fertile eggs transferred basis ranged from 73.02 to 83.41% (S<sub>28</sub>). An overview of the comparative performance for various economically important traits also reflected an





### Random Sample Poultry Performance Test (RSPPT)

The broiler crosses from four AICRP centres on Poultry Breeding participated in the 24th and 25th Random Sample Poultry Performance Tests (RSPPT), held at Gurgaon. The body weight of different commercial crosses of AICRP strains at 6 and 7 weeks varied from 1,262 to 1,436 g and 1,692 to 1,924 g, respectively. The feed conversion ratio (FCR) up to 6 weeks was around 2.0. The cross Krishibro ranked third in overall performance among all the entries in the 24th RSPPT.

improvement over preceding generation ( $S_{27} > S_{26}$ ). The main trait under selection (64 week EP) improved to the tune of 25 to 37 eggs in the pure strain and 42 eggs in HI strain cross ( $191.48 < 216.43$  in IWH;  $172 < 209$  in IWI;  $184 < 225$  in HI). The average genetic responses per generation for 64th week of egg production were significant (1.11 and 1.36 eggs in IWH and IWI strain, respectively).

In HR (crossbred) the average egg production up to 64 weeks of age was 213.67 eggs with 51.77 g of egg weight. The corresponding figures for CD cross were 167.21 eggs and 55.30 g. The body weight and egg weight at 64 weeks of age in HI (strain cross) were 1,437 g and 52 g respectively. The egg production up to 64-weeks of age on HH and HD basis were observed as 221 and 225.25 eggs respectively.

The mean performance of RIR ( $S_{24}$ ) for 40-week egg production was 101.63 eggs with 52.20 g of egg weight and 5,296.31 g of egg mass. The CARI Sonali (brown egg commercial layer), secured second position in RSLT at Gurgaon.

### Poultry for meat

Under the AICRP on Poultry Breeding, six broiler pure lines were improved, with mass selection for 5-week body weight giving due weightage for conformation traits in the male line, and 5-week body weight, egg production and hatchability in the female

Colour broiler male line



lines with the ultimate objective of developing colour broiler crosses for commercial farming. The females matured at 180 days of age and laid 71.0 eggs till 40 weeks. Body weight at 5 weeks of age was 920 g and FCR was 1.93. The S-30 generation of PB-2 population was also evaluated. The fertility and hatchability on total eggs set were 94.2 and 88.8%, respectively. In comparison to the previous generation, body weight at 5 weeks (872 g) improved by 48 g and egg production (121 eggs) was higher by 4 eggs up to 52 weeks in the line. The S-10 generation of SDL population was evaluated at Bhubaneswar. The intensity of selection was 1.09  $\sigma$  for males and 1.01  $\sigma$  for females. The fifth week body weight of this population in the current generation was 1,123 g. The feed conversion ratio up to 5 weeks was 1.92. The SDL population matured 2 days earlier than the previous generation.

In the current generation, intensity of selection was 1.59  $\sigma$  in CSML (Colour synthetic male line) and 1.53  $\sigma$  in CSFL (colour synthetic female lines). The fertility was 82.2 and 79.8% in CSML and CSFL, respectively. The fifth week body weight of CSML and CSFL in the current generation was 1,069 and 1,056 g, respectively. The phenotypic response per generation for 5-week body weight was 30.2 and 34 g, and the corresponding genetic response was 20.4 g and 24.2 g, respectively, in CSML and CSFL in the last five generations. The females of CSFL line matured 10 days early and produced two eggs more over previous generation. At the JNKVV, Jabalpur, development of purebred dwarf dam line (white and colour lines) (G-7 generation) was undertaken. Body weight at 6 weeks, egg production and hatchability were considered for improvement. The intensity of selection was 1.09  $\sigma$ . The per cent fertility was 89-90%. Hatchability on total eggs set increased and body weight at 6 weeks of age (679 and 856 g in colour and white lines, respectively) improved by 74 g over previous generation.

Synthetic colour broiler breeder populations (PB-1 and PB-2) were improved for traits of economic importance in broiler production. The cross of the two lines, a multi-colour commercial broiler, named as Krishibro, has become popular for intensive farming on low input requirement in areas having special market demand for colour broilers. The selection differential for 5 week body weight was 113 and 117.4 g in male and female lines, while the intensity of selection was 1.30 and 1.41  $\sigma$ , respectively. The fertility and hatchability on fertile eggs set were 73.1 and 87.4%, respectively, in male line, while the corresponding data for the female line were 71.5% for both the traits. The control population maintained simultaneously was stable for the important traits.

**Tropical broiler production:** The naked neck and dwarf gene populations, developed on broiler background, were maintained at the PD on Poultry, for use as resource populations for use in tropical broiler breeding programmes. Both the lines were evaluated for juvenile and early production traits in S-4 generation.





### Economic gain in tribal areas

Backyard poultry is popular with the tribal community, but they never bother for the loss or gain from such poultry farming. The economic aspect of backyard poultry farming were assessed at their doorstep and following data were recorded:

- The sale of the eggs is restricted even though the price is higher as compared to the commercial eggs available in the market as these eggs are utilized for multiplication.
- The cock and pullet are sold @ Rs 75 to Rs 120/kg live weight, when they need money to purchase their daily ration and to meet their emergency.
- The average income from single hen accounts about Rs 600/year.



In naked neck gene line, fertility was 84.2% and hatchability on total and fertile eggs set was 67.1 and 79.7%, respectively. The body weight at 6 weeks was 877.6 g. The production traits like age at sexual maturity (ASM), body weight at 20 and 40 weeks, egg weight at 40 weeks and egg production up to 40 weeks were 175.8 days, 2,349 g, 3,158 g, 61.5 g and 62.6 eggs, respectively. In dwarf gene line, fertility was 76.6% and hatchability on total eggs set was 69.7%. The body weight at 6 weeks was 607.3 g. The ASM, body weight at 20 and 40 weeks, egg weight at 40 weeks and egg production up to 40 weeks in dwarf line were 151.5 days, 2,104 g, 2,563 g, 56.6 g and 59.3 eggs, respectively.

Among synthetic broiler male lines, fertility percentage was 89.20, 88.11 and 74.13 in CSML, SML and control lines respectively. Percentages of hatchability were 86.75, 86.51 and 84.70, respectively, in CSML, SML and control lines. The overall average of body weight at 3 and 5 weeks in corresponding lines were  $580.18 \pm 0.97$  and  $1,068.77 \pm 2.78$  g,  $521.31 \pm 3.45$  and  $1,060.34 \pm 6.58$  g and  $387.54 \pm 2.94$  and  $751.57 \pm 5.25$  g, respectively. The overall average of body weight at 3 and 5 weeks in CSFL were  $553.10 \pm 1.54$  and  $1,056.28 \pm 2.96$  g, respectively. The average egg production up to 40 week were 61.2 eggs in CSFL and 62.8 eggs per bird in SDL. Fertility percentage in IC3, IR3 and CARIBRO Tropicana were 77.70, 89.54 and 84.69, respectively.

### Development of germplasm for rural poultry farming

Specialized male and female lines with colour plumage were developed for production of varieties, Vanaraja (dual purpose) and Gramapriya (egg type) for rural poultry farming. In Vanaraja male line, emphasis was given for improvement of shank length, while no intentional selection was practiced for juvenile body weight. In S-7 generation, the body weight, shank length and SRBC titre of this line at 6 weeks of age were 595 g, 71.3 mm and 6.7  $\log_2$ , respectively. The production traits like ASM, body weight at 20 and 40 weeks of age, egg weight at 40 weeks and egg production up to 40 weeks were 178.4 days, 1,881 g, 2,789 g, 56.1 g and 42.2 eggs, respectively.

The Vanaraja female line was improved for egg production. In S-5 generation, ASM, body weight at 20 and 40 weeks of age, egg weight at 40 weeks and egg production up to 40 weeks were 170.1 days, 2,141 g, 2,512 g, 53.8 g and 63.4 eggs, respectively. In S-6 generation, body weight, shank length and



Vanaraja male line



Dwarf Gene and Naked Neck lines were used for tropical broiler production



Vanaraja female line



Dahlem Red line

SRBC titre at 6 weeks were 723 g, 76.2 mm and 6.5 log<sub>2</sub> units, respectively.

Dahlem Red population was developed as a female line for production of Gramapriya, for backyard poultry production. The age at sexual maturity, egg weight, egg production and egg mass to 40 weeks were 175 days, 56.2 g, 75.1 eggs and 4.45 kg, respectively.

**New promising crosses for rural poultry production:** The performance of two new crosses, C1 and C2 was evaluated along with Vanaraja and Gramapriya under intensive system. Both the crosses excelled over Vanaraja on all the growth and production parameters up to 40 weeks of age. The C1 cross excelled over Gramapriya and Vanaraja both in body weight and egg production. Moreover, the C1 cross recorded optimum juvenile body weight (about 600 g at 6 weeks), shank length (71.14 mm), better egg weight and production, which are suitable for backyard dual purpose variety. However, C2 cross was superior in body weight at 6 weeks of age and age at sexual maturity.

## Duck

**Native ducks:** The morphological and production traits of ducks were recorded. The body weights of native ducks were 150–200 g less compared to Khaki Campbell. The mature body weights

## White quail

A recessive auto sexing white-feathered quail line was developed. The broiler quail line CARL Uttam achieved fifth week body weight of  $196.67 \pm 2.84$  and  $204.88 \pm 2.89$  g, for males and females, respectively. The layer quail line CARL Pearl recorded fifth week body weight of  $121.34 \pm 2.41$  and  $128.17 \pm 2.86$  g for males and females, respectively. The base populations of CARL-Brown have been established. The fifth week body weight in this line in male was  $170.18 \pm 2.86$  g while the corresponding value from female was  $186.28 \pm 2.89$  g.

of male and females are 1,400–1,500 and 1,200–1,300 g. The recorded egg weight was 65–75 g. They usually lay 200–220 eggs/year in intensive and free-range system of management.

## Evaluation of native, exotic ducks and their crossbred:

The hatchability percentage on total egg set basis in Khaki Campbell, *desi*, White Pekin, KD and DK were 66.01, 52.22, 65.31, 48.20 and 48.02%, respectively.

Body weight of White Pekin replacement ducklings at seventh and eighth week of age in male and female were  $2,010 \pm 20.17$ ,  $2,107 \pm 21.33$  g and  $1,996 \pm 19.76$ ,  $2,019 \pm 18.3$  g, respectively. Mortality per cent in combined sex from 0–8 week of age was 4.62%. Body weights of female at 16 and 20 weeks of age were  $3,038 \pm 26.45$  and  $3,114 \pm 25.95$  g, respectively. The HD egg production per bird up to 36th week of age was 41.06 eggs. The egg weight at 36th week of age was  $75.81 \pm 0.58$  g. The HD egg production up to 72 week of age in the old stock of White Pekin was 172.06 eggs. The HD egg production per bird in Khaki Campbell up to 72 weeks of age was 239 eggs.

**Comparative evaluation of Khaki Campbell (K), *desi* (D), KD and DK crossbred:** Statistically significant differences were observed between different genetic groups for body weight at different ages. The 16th week body weight in K, D, KD and DK were  $1,373 \pm 7.94$ ,  $1,450 \pm 8.70$ ,  $1,292 \pm 14.55$  and  $1,288 \pm 15.27$  g, respectively. The best FCR up to sixth week of age was obtained in K (2.923) followed by KD (3.315), DK (3.350) and D (3.664). The hen day egg



Moti (Malkangiri) ducks



The fawn type runners



Ducklings





production up to 36th week of age was highest in DK (64.35 eggs) followed by KD (52.95 eggs), D (50.90 eggs) and K (49.52 eggs). Eggs weight at 36th week in K, D, KD and DK were  $63.97 \pm 0.14$ ,  $67.38 \pm 0.43$ ,  $68.76 \pm 0.59$  and  $68.24 \pm 0.45$  g, respectively.

## ANIMAL HEALTH

### Foot and mouth disease

During the year 2,852 FMD field outbreaks were recorded in the country and 2,257 samples were analyzed for serotyping by sandwich ELISA. Type identification could be made in 653 samples comprising 532 type O, 63 type A and 58 type Asia1. No virus could be detected in about 51% of the samples. Like the previous years outbreak due to type O predominated, and outbreaks due to type O, A and Asia1 virus were recorded in 19, 8 and 8 states, respectively.

Large majority of field isolates of types O and all the isolates of Asia 1 are antigenically related to the vaccine strains currently in use, indicating that the vaccine strains are good enough to cover the circulating field isolates. However the data generated for type A for this year also corroborates our earlier observation that in type A the field situation demands a change of vaccine strains. Different field strains are being characterized as a candidate vaccine strain replacement for type A.

Molecular epidemiological analysis of type O virus indicated continued dominance of Pan Asia III strains during this year also. However in Asia 1, lineage CII takes the center part after a gap of 4 years. The lineage VII which had been the major force behind type A outbreaks in the recent years continues to retain the prime position. One of the major observations of the year is that FMD returned to Andaman and Nicobar islands after 16 years of absence. Investigation revealed that infection had gone from the mainland and Pan Asia lineage of type O virus that circulated southern India during 1998–2002 was responsible for this outbreak.

A study undertaken to shortlist few more candidate vaccine strains in type O, indicated that isolates IND 271/01 and IND 120/02 could be ideal substitutes to current vaccine strain IND R2/75. Complete nucleotide sequence of all the short listed strains were generated, similarly, the complete nucleotide sequence of several field isolates of type Asia 1 was determined. The tree topology and

- FMD was reported from Andaman and Nicobar islands
- PCR developed for determination of lineage of the type Asia 1 field isolates
- PD\_ADMAS detected BHV-1 genome in peripheral blood leucocytes, aborted materials etc.
- Blue tongue virus vaccine yielded promising results in local and Bharat Merino sheep
- A project on Development of Economic Analysis of animal diseases and their control was initiated
- H5N1 strain of influenza A virus was detected in poultry for the first time in country
- RT-PCR developed for diagnosis of border disease virus infection
- Herbal medicine found effective in clinical case of diarrhea
- *In ovo* vaccination in broiler chickens was standardized
- Seroprevalence of rotavirus antibody was studied in mithuns

clustering pattern was similar when complete coding region or primary poly-protein cleavage products in isolation were used for analysis. The identification of residues under positive selection, of which some are antigenically critical, made a beginning in the understanding of antigenic features of this serotype.

A PCR was developed to determine the lineage of the type Asia 1 field isolates involved in the outbreaks. This PCR gives a fast preliminary indication on the genetic lineage before proceeding with nucleotide sequencing of the 1D region of the viral genome, which definitely continues to be the confirmatory method to assign lineages by phylogenetic analysis. This assay promises to be an effective tool in molecular epidemiological investigation of FMD in the country.

Detection of foot-and-mouth disease virus (FMDV) from clinical specimens by conventional sandwich enzyme-linked immunosorbent assay (ELISA) and virus isolation in cell culture is often compromised owing to limited sensitivity and sample deterioration during transit. An RT-PCR (oligoprobing) ELISA in both solid and liquid phase hybridization formats targeting an across serotype conserved site at 3C–3D region was developed and its effectiveness was compared with that of the known targets at the IRES region. A non-isotopic RNA dot hybridization assay with colorimetric detection targeting both the IRES and the 3D region, was also validated, which is capable of handling high throughput samples with ease. RT-PCR (oligoprobing) ELISA and dot hybridization assay showed 1,000- and 10-fold greater sensitivity than the sandwich ELISA, respectively.

Evaluation of various extraction protocols for RNA isolation revealed that sample preparation in DEPC-water/PBS may not be ideal for it as compared to their preparation in lysis buffer prior to RNA extraction. Virus specific primer for reverse transcription of the RNA was more efficient compared to oligo dT or random primers.

### PCR protocol for *Brucella* detection

PCR protocol to detect *Brucella abortus* and *B. melitensis* directly from the aborted material, foetal contents and vaginal discharges was standardized. The direct detection of *Brucella* using sensitive PCR is advantageous over isolation and serological tests and risk of laboratory acquired infection can be avoided. The standardized protocol can be used to screen suspected herds for confirmatory diagnosis.





### Databases to study epidemiology of animal diseases

To study the national epidemiological status of economically important animal diseases, the following databases were collected, updated and formatted.

- Livestock demography at district level
- Livestock disease profile at district level
- Comprehensive district level censuses data 2003 developed as RDBMS
- National meteorological database
- Agro-ecological data—The country has been classified into 20 macro zones for the convenience of epidemiological analysis
- Development of GIS Admin level I and II level polygon and development of point projection maps
- National sampling frame of villages (epi\_units) for epidemiological studies updated as on 2006

Functional livestock disease relational database software supported by GIS NADRES is made available on the internet. This website is available to general users such as administrators, planners and farmers. It is an interactive website through which animal disease forecasting, veterinary web pages on important animal diseases, epidemiological reports and maps are available. The disease forecasting module is updated regularly and with this the probability of outbreak of a specific disease at district level can be predicted two months ahead.

Plasmid vector, expressing short hairpin RNAs (shRNA) under mouse U6 and CMV promoters directed against a highly conserved sequence in the 2B-2C nonstructural protein-coding region of FMDV RNA identified earlier, were transfected to BHK-21 cells. At different quantum of infection (10 to 1,000 TCID<sub>50</sub>), these cells showed a decrease in virus yield as observed in ELISA and virus titration when compared to control cells. The level of inhibition varied among the serotypes of virus, between the promoters used and the time of infection.

The virus repository is one of the big collections of cell culture revived field strains. The National repository contained 1,250 (800-O, 235-Asia 1, 200-A, 15-C) field isolates.

### Animal disease monitoring and surveillance

National sero-epidemiological surveys conducted for brucellosis and infectious bovine rhinotracheitis (IBR) revealed that overall incidence was 22.4% in 14 states 37% in nine states.

### Epidemiological studies

**Brucellosis:** Standardizations of latex based agglutination (LTA) for the detection of brucella antibodies in cattle, sheep and goat was standardized using sLPS, heat killed and sonicated antigens of *Brucella abortus* S<sub>99</sub> strain. Sensitivity and specificity of the test are being evaluated in comparison with other tests.

Indirect-ELISA protocol for brucellosis was standardized for small ruminants. Screening for *Brucella* antibodies from sera samples from small ruminants by RBPT, STAT and iELISA revealed higher seroprevalence of brucellosis in sheep (13.41%, 8.23%, and 9.14%) than in goats (8.27%, 4.43%, and 6.49%), and prevalence was the highest in Gujarat (26.08%, 17.30%, and 22.6%) followed by Karnataka (14.93%, 7.23%, and 7.23%) and lowest in Rajasthan (5.53%, 4.11%, and 4.78%). Seropositivity was found the highest by RBPT (9.95%) followed by iELISA (7.36%) and least in STAT (5.67%). The brucellosis prevalence rate by all the three serological tests was more than 5%. The test appears to be promising for large scale screening of sera samples.

**Molecular epidemiology of *Brucella* strains:** *alk B* gene in chromosome II region of *B. abortus* (498 bp) was cloned and sequenced. Comparison of this nucleotide sequence of *B. abortus* S<sub>99</sub> strain with those of reference sequences from USA (GenBank No. AE017224, AM040265 and AF148682), revealed 100% homology with all the three sequences. Similarly, the PCR amplified product of hypothetical protein in chromosome I of *B. melitensis* 16 M strain (731 bp) was also sequenced and nucleotide sequences were aligned with two reference sequences from USA (GenBank No. AE 009614 and AE009540) and one from Belgium (AF047478). There was 98.8% homology with all the three sequences.

**Infectious bovine rhino-tracheitis (IBR):** Screening of serum samples of bovine population from nine states of India by using AB-ELISA kit, revealed an overall antibody prevalence of 36.66% against BHV 1. The highest prevalence was in Meghalaya (59.09%) and lowest in Tamil Nadu (24.57%). The main means of control is elimination of carrier bulls. BHV 1 genome detection in peripheral blood leucocytes, aborted materials, semen samples etc. have already been undertaken.

BHV 1 infection in bulls is epidemiologically important as the virus is excreted intermittently in the semen and may cause spread of infection to all other cattle who receive semen from such breeding bulls. Identification and elimination of the bulls positive for BHV 1 antibodies and antigens by serological tests and PCR assays, respectively, would help in formulating the control strategies. An initiative to develop a PCR based detection of BHV 1 genome/antigen in the semen samples of all bulls in the breeding farms on regular basis, was initiated.

**Molecular epidemiology of BHV 1 in India:** The long term serological studies showed evidence of the disease all over the country, although there are only limited reports of isolation of virus mainly from Karnataka, Punjab and Uttar Pradesh. Pilot studies are being carried out using blood in EDTA collected from different cattle showing the symptoms of repeat breeding, abortions etc. The total DNA was extracted from PBL and was subjected to PCR using different primers specific for gB, gC, gD of BHV 1.



### Serum based IBR AB-ELISA kit

Ever since the release of indigenously developed, software based AB-ELISA kit, there has been a growing demand for this kit from all over the country. The sale of kits also includes hands-on-training and help in times of troubleshooting. The kit is user friendly, economical and provides high levels of specificity and sensitivity. The sale of kits priced at Rs 25/test sample and supplied in 500 test capacity generated an appreciable income.

Comparison of the gB sequence of Indian isolates with those of reference sequences (one from Switzerland and two from Brazil), revealed that there was 100% homology with the sequence of AJ 004801 (Switzerland), 98.3% homology with AY 58382 and 98.7% homology with AY 330349 (both from Brazil). In clustering (rooted phylogenetic tree), Indian isolate was closer to the sequence AJ 004801 (Switzerland) and AY 330349 (Brazil).

**Trypanosomiasis:** The detection of carrier status of *Trypanosoma evansi* is a challenge in field condition as the carrier animals became nuclei for propagation of the disease in a particular area. Variable surface glycoprotein (VSG) which possesses diagnostic importance is having tremendous antigenic diversity, hence conventional polyclonal ELISA is not sufficient enough to detect the infection (carrier status). PCR technique was developed to detect the carrier status (latent infection) in domesticated animals. A pair of primers specific to VSG gene of *T. evansi* was developed.

**Peste des petits ruminants (PPR):** A national survey mainly comprising the states of Southern peninsular India and western states was conducted for seroprevalence. Screening of serum samples from sheep (1,492) and goats (2,423) from seven states revealed 41% seroprevalence in sheep and 32% in goat.

**Leptospirosis:** *Leptospira* species could be isolated from 15 (25%) out of 62 cases of bovine abortions. *Leptospira* staining kit is ready for commercial use.

### Demographic studies on animal diseases of economic importance

Based on the reported incidence of the diseases over past 15 years, an epidemiological analysis of bacterial, viral and parasitic diseases of economic importance was worked out. Among the bacterial diseases, haemorrhagic septicaemia tops the list and the occurrence of the disease is throughout the country.

**Blue tongue disease:** Isolates of bluetongue virus types 1 (from Hisar and Chennai), 2 (from Hyderabad), 9 (from Hyderabad), 15 (from Hyderabad and Kolkata), 18 (from Parbhani and Bangalore) and 23 (from Izatnagar, Bangalore and Chennai) were confirmed and submitted to virus repository at IVRI,

Mukteswar. Fifteen BTV isolates (6 from Izatnagar, 4 from Hyderabad, 2 from Hisar, 1 from Parbhani and 1 from Bangalore) were deposited in the repository and are being maintained. The epidemiology of the last 5-6 years information revealed no outbreak of bluetongue in Rajasthan except in the year 2001–2002, which may be attributed to prolonged drought in the state. Andhra Pradesh, recorded most severe outbreak only during 2005 with 880 outbreaks with maximum attacks and deaths whereas outbreaks are being reported regularly from Karnataka and Tamil Nadu.

Seroepidemiology revealed an incidence as high as 70% in sheep, 85.33% in goat and 66.12% in bovine in Rajasthan. In Gujarat also higher percentage of seroprevalence of BT in goats (45.32), cattle (48.39) and buffaloes (44.67) than in sheep (36.12) and camel (35.63), was observed. Out of 433 serum samples from 6 districts of Madhya Pradesh tested, goat (24.5%), cattle (20.43%) and buffalo (19.35%) samples revealed the presence of BT antibodies. None of the sheep (21) or *cheetal* (38) sera was positive for antibodies. Andhra Pradesh showed per cent seropositivity of 35.39, 33.47, 9.09, and 8.25 in sheep, goats, cattle and buffaloes, respectively. Maharashtra had an incidence of 41.95% in sheep, 42.91% in goats, 42.85% in cattle and 84.61% in buffaloes. Karnataka had incidence of 42.39% in sheep, 27.48% in goats and 18% in cattle, whereas West Bengal showed an incidence of 31.12% in sheep, 22.28% in goats and 15.97% cattle.

Monoclonal antibodies produced against BTV reacted well with BTV r-Ag and purified BTV antigen. In indirect ELISA 9 out of 23 clones gave maximum absorbance.

**Vaccine development:** BTV vaccine using BEI inactivated bluetongue virus serotype 1 adjuvanted with saponin or seppic oil yielded promising results in local and Bharat Merino breeds of sheep.

**Identification of vector species:** Samples of midges from Uttar Pradesh, Uttarakhand, Gujarat, West Bengal, Haryana, and Rajasthan were identified as *Culicoides oxystoma*, *C. clavipalpis*, *C. actoni*, *C. anophelis*, *C. orientalis*, *C. similis* and *C. imicola*.

**Typing of new isolates:** Type specific primer designing for typing of new isolates of BTV, development of multiplex RT-PCR for differential diagnosis of BTV and PPRV, RNA-PAGE of the

### National Serum Bank

The PD\_ADMAS has established a National Serum Bank Facility with 166,037 serum samples collected from different States and Union Territories including 70,904 samples from cattle, 32,599 from buffaloes, 10,972 from sheep, 51,491 from goat and 71 from pigs. The serum samples were computer catalogued, stored at –20°C to –80°C and used for testing the presence of antibodies against different diseases like RP, PPR, IBR and brucellosis.



isolates, nucleotide sequence studies to determine the relationship of Indian isolates with other global isolates, were done. BTV-2 isolate from Hyderabad was found more closely related to European and Chinese isolate than to American isolate. Homology among Indian and Australian isolates ranged from 95.7 to 99.4%. Indian BTV types are closely related to each other forming monophyletic group.

A panel of 23 hybridoma clones reacting to purified BTV 23 antigen in indirect ELISA was produced. Out of 23 clones, 11 clones showed reactants with purified recombinant VP7 protein of BTV.

**Gastro-intestinal parasitism:** Epidemiological studies conducted in different agro-climatic zones of different states revealed various parasitic infections. In goat the infection rate was 56 to 70% with highest percentage in Madhya Pradesh followed by West Bengal and Sikkim. In sheep the rate of infection was 49.5 to 80% with highest rate in Madhya Pradesh than West Bengal (56%) followed by Meghalaya (55.00%) and Tamil Nadu (27.3–34.9%) however, in Uttarakhand comparatively a lower rate was recorded (12.43%) in cattle. In buffaloes a similar trend of infection was recorded. In Sikkim the infection rate ranged between 9.72 to 28% in yak. In Meghalaya the infection rate in pig was 33.5%. It was observed that both young and adult animals are infected with GI nematodes. In all the states of the country *Haemonchus contortus* was found to be the predominant infection in all the ruminant species. Bioclimatograph has been prepared for the semi arid and arid regions in Rajasthan. A software 'FROGIN' has been developed for forecasting the intensity of infection in sheep particularly *H. contortus* for semi arid region. The performance of FROGIN programme was evaluated in both the agro-climatic zones of Rajasthan, which gave precise prediction for gastro-intestinal nematode infection. In dot-ELISA affinity purified fraction of ES antigen recognized *H. contortus* infection at 4 day PI indicating diagnosis of preclinical infection. In western blotting, 60 and 120 kDa polypeptides of ES antigen were recognized as early as 4 day PI by the experimental sera indicating these to be immunodominant polypeptides. Immunoaffinity purified fraction of ES antigen also recognized 120 kDa polypeptide as early as 4 day PI. In the larval antigen 60, 120 and 170 kDa polypeptide have been recognized by 4 day experimental sera of sheep in western blotting indicating diagnostic value. In ELISA, dot-ELISA and western blotting cross antigenicity was recorded between *H. contortus* and *Oesophagostomum* spp. 60 kDa polypeptide has been determined to be glutamate dehydrogenase biochemically. In *Oesophagostomum*, 31 kDa and in *Bunostomum*, 152 kDa polypeptide have been found to be immunodominant in western blotting. Work on the production of recombinant antigen H-gal-GP and H-11 are under progress for immunoprophylaxis. PCR based technique was developed for detection of benzimidazole resistance in *H. contortus* in farm and

### Quality control and production of veterinary biologicals

A total of 178,000 doses of RD 'F' strain vaccine, 550,000 doses of RD 'Mukteshwar' strain vaccine, 110,000 doses of fowl pox vaccine, 55,550 doses of lapinized swine fever vaccine, 99,100 doses tissue culture sheep pox vaccine, 11,280 ml of H.S. oil adjuvant vaccine, 2,630 doses of *Brucella abortus* strain-19 (live) vaccine, 500 doses of *Brucella melitensis* Rev-1 vaccine, 600 doses enterotoxaemia vaccine, 100,000 doses of tuberculin PPD, 3,500 doses of avian PPD, 96,500 ml of *Brucella* agglutination test antigen, 7,080 ml of *Brucella abortus* Bang Ring antigen, 13,780 ml of Rose Bengal Plate Test antigen, 150 ml of *Brucella* positive serum, 2,500 ml of *Salmonella Abortus equi* 'H' antigen, 57 ml of *Salmonella* poly 'O' sera, 3,500 ml of *S. Pullorum* plain antigen and 6,220 ml of *S. Pullorum* coloured antigen, were produced and quality tested. A total of 143,100 doses of tissue culture sheep pox vaccine was tested for infectivity titre. A total of 59.9 lakh doses of PPR vaccine were produced and supplied to the Uttar Pradesh, Animal Husbandry Department. Using FMD vaccine production technology in BHK 21 cell culture, a total of 13.97 million doses of monovalent FMD vaccine were produced.

field samples. *Duddingtonia flagrans*, a nematophagous fungi has been evaluated for biological control of GI nematodes and predatory activity of the fungi and its gut passage survival was also determined.

**Haemorrhagic septicaemia:** A total of 58 *Pasteurella multocida* isolates from various animal species and birds were characterized using morphological, cultural, biochemical studies. All the isolates were found pathogenic to mice. Most of the isolates were sensitive to chloramphenicol, cloxacillin, ceftioxone and resistant to co-trimoxazole, oxytetracycline and erythromycin. Molecular studies were conducted for identification, characterization of *Pasteurella multocida* isolates from various animals and avian species by PM-PCR, HSB-PCR, Multiplex-PCR, RAPD-PCR and REP-PCR. All the isolates of *Pasteurella multocida* were typed as capsular group B. Cloning and sequencing of 16S RNA gene from different isolates of *Pasteurella multocida* B : 2 recovered from various animal species revealed 99.9% homology among the isolates of cattle, pig and sheep whereas, goat isolates shared 99.8% homology. Outer membrane protein from *Pasteurella multocida* B : 2 serotype were isolated and characterized by SDS-PAGE and western blotting. Two major proteins of 32 kDa and 38 kDa were observed and found to be immunogenic in rabbits. Low volume saponified HS vaccine gave 100% and 80% protection in cow calves at 9 and 12 months post vaccination, respectively, on direct challenge test. A preliminary trial of combined HS and FMD vaccine (virus type A, O and Asia 1) with a new adjuvant (seppic)





### Technology assessment, refinement and transfer

A simple dot-ELISA using nitrocellulose membrane as solid support was developed for detection of PPR viral antigen in caprine and ovine clinical materials for easy and early diagnosis. Standardization of dot-ELISA using cell culture adapted PPR vaccine virus at different temperatures (37°, 42° and 45°C) of incubation, revealed that there was no difference in the detection of PPR viral antigen in relation to the incubation temperatures. The standardized dot-ELISA with known virus was well adapted for the detection of the known positive and negative clinical samples, unequivocally indicating that the test can be used for diagnosis of PPR in field at different temperatures. The validation of the assay was assessed with a variety of field clinical materials obtained from sheep and goats from various geographical locations of the country. The dot-ELISA detected PPR virus antigen in 149 out of 320 caprine materials giving a percentage positivity of 47, and the S-ELISA detected 154 out of 320 samples with percentage positivity of 48. The diagnostic sensitivity and specificity of dot-ELISA were found 82% and 91%, respectively, as compared to S-ELISA. However, the S-ELISA could not detect the viral antigen in all the positive samples, as the detection limit was about 500 TCID<sub>50</sub>/ml. There was agreement between dot-ELISA and S-ELISA. Dot-ELISA has the advantages of being simple, easy to assay the results and perform under field conditions at various temperatures of incubation thereby fulfilling the criteria of an ideal pen side test for the diagnosis of PPR.

was carried out at IVRI Bangalore campus, Immune response of this combined vaccine was observed on 28th day post vaccination by direct challenge and the animals withstood the challenge test.

### Exotic and emerging diseases

H5N1 strain of influenza A virus was detected in poultry for the first time in the country as a result of comprehensive field and laboratory investigations of clinical samples collected/received from Nandurbar, Jalgaon districts of Maharashtra, Uchchal of Gujarat and Burhanpur district of Madhya Pradesh. Four H9N2 and one H9N1 viruses were isolated. Porcine parvo and circovirus-1 genomic sequences were detected in the tonsillar epithelium of Indian pigs. Prevalence of antibodies to pestiviruses (border disease virus and bovine viral diarrhoea virus) was found in sheep and goats for the first time in India. More than 40 pestiviruses were isolated from sheep and goats and BVDV type 2 were detected in goats. Real-time PCR assays were standardized for typing H5 sub-typing of avian influenza virus. An anti-BIV gag recombinant phage library was developed as an alternative to monoclonal antibodies produced by hybridoma technology. An RT-PCR for diagnosis of border disease virus infection in small ruminants and a nested PCR were developed for differentiation of BDV, BVDV type 1 and BVDV type 2.

### Disease diagnosis

Two Indian Orf virus isolates, one each from ovine and caprine, isolated in primary/secondary lamb testes cell culture for the first time were confirmed by using EM and semi-nested diagnostic PCR. Presence of G2 genotype of *Ecchinococcus granulosus* in Indian water buffalo is the first report from India.

### Vaccines

Field trial of live attenuated **goat-pox vaccine** carried out in Maharashtra, Tamil Nadu, Karnataka, Orissa and West Bengal, showed complete protection for two years upon challenge. Sandwich ELISA evaluated for its usefulness as a quality control test for **PPR vaccine** was found suitable for rapid screening of vaccine batches for field use, showing good correlation with the conventional virus titration assays. Incorporation of groundnut oil with **ND vaccine** and its combination with sunflower oil administered with rabies DNA vaccine enhanced immunogenicity of these vaccines. Inactivated oil emulsified vaccine prepared from concentrated **CIA virus** induced good immunity. **Turkey pox virus** attenuated by passaging in chicken embryos provided better protection in comparison to tissue culture passaged virus. The 39 KDa gene was found useful in detecting vaccination among **avian pox virus**. The field trials of live **Salmonella Abortus equi vaccine** in young mares revealed that the vaccine is safe for use in young animals. The recombinant L7/L12 ribosomal protein of **Brucella abortus** and its DNA vaccine were found completely safe in pregnant guinea pigs.

### Diagnostics

The recombinant “H” protein of PPR virus expressed constitutively in vero cells can serve as virus antigen in **PPR** competitive ELISA and may replace the whole virus antigen in use currently. An ELISA was standardized using recombinant protein coded by VP2 gene for detection of antibodies in chickens against **IBD virus**. A multiplex PCR was standardized targeted to four virulence genes, viz. *blyA*, *actA*, *plcA* and *iapA* of **Listeria monocytogenes** and used for studying profiles of the isolates from human spontaneous abortion cases. PCR assays were standardized and used for detection of genus **Campylobacter** from faeces, semen and preputal washings, and **Campylobacter fetus** from semen and preputal washings. An outer membrane protein (OMP) based tube agglutination test was developed for the identification of **Aeromonas** cultures. PCR based method to differentiate cattle and buffalo tissues was standardized for **forensic application**. A PCR test was optimized for the **genus level diagnosis** and molecular identification of **Haemonchus contortus**. Cadherin and fibronectin, immunohistochemical molecular markers, caused different labeling and expression in



benign and malignant **tumours** of animals, suggesting their application in characterization of these tumours.

### Herbal medicines

The modified DS formulation was highly effective in clinical cases of diarrhoea. The formulation given twice daily for 3 days afforded complete cure from severe **diarrhoea** in male cattle and buffalo. Oral electrolyte solution with high energy and glutamine was most effective supportive therapy in mild to moderate **calf diarrhoea** closely followed in terms of efficacy by oral formulation containing powder of *bael* fruit, *shisham* leaves, glucose and glutamine. In decreasing order of efficacy are two combinations (i) powder of *bael* fruit, *shisham* leaves and glucose, (ii) powder of *bael* fruit and *shisham* leaves. Zn, Se and vitamin E supplementation reduced **oxidative damage** by scavenging free radicals and proved beneficial against **stress due to diarrhoea**. Zinc @ 10.5 mg/kg body weight once daily for 5 days along with standard antibiotic was the best **antioxidant** in diarrhoea in farm animals. *Curcuma longa* @ 1.5 g/kg b.wt. administered orally for 3 days along with Zn as an antioxidant was found highly effective in **diarrhoea** in calves. Mixture of oil of *Jatropha curcas* along with zinc, selenium and *Curcuma longa* showed high therapeutic efficacy on local application against **canine demodicosis**. These adjunct therapies enhanced recovery rate from oxidative stress in infected canines. *Phyllanthus emblica* and *Curcuma longa* mixed extract therapy was found better immuno-enhancer than *Allium sativum* in treatment of **mastitis** in cattle. Cystic irrigation with ammonium chloride was found to be a good technique for **dissolution of urethral calculi** and the combination of *Pasanbhed* and *Gokbru* has the potential for dissolution of urethral calculi. Various essential oils from plants namely, EOIVR-2&3 and EOIVR-6&7 completely **paralyzed** *Gastrothylax crumenifer* and *Haemonchus contortus* and *Fasciola hepatica* and *Gigantocotyle explanatum*, respectively, *in-vitro*.

### Surgical and clinical interventions

Techniques for the correction of **ante bracheal deformities** in growing dogs affected with skeletal diseases were developed. Surgical excision followed by chemotherapy with vincristine sulphate and cinnoline proved to be an effective method for the treatment of **canine mammary tumours**. A modified technique of interlocking nailing was successfully used for the **management of fractures** in osteopenic bones in small animals. The clinical and laboratory investigation revealed that oral administration of **meloxicam** was safe for vultures and four other species of scavenging birds. Gestational stages had a significant influence on both myometrial and placental COX-1 and COX-2 activities.

### Immunogenicity of IVRI FMD vaccine under field conditions in Karnataka

Serum samples collected from the previously vaccinated animals in various villages were tested for the serum neutralizing antibody titers against type 'O' and 'A' and type 'Asia 1'. The data on SN antibody titers revealed that a minimum of four FMD vaccinations are required for developing the herd immunity, as less number of vaccinations did not maintain significant SN antibody titers (SN log 10 titers of less than 1.5).

Especially, the changes in uterine enzyme activity may have an influence on the **myometrial contractility** during different stages of gestation through altered prostaglandin synthesis. The **non-steroidal anti-inflammatory** drug nimesulide exhibited favourable pharmacokinetic properties including long elimination half-life and mean residence time and better bioavailability following intramuscular administration and thus, the drug has therapeutic potential for use by intramuscular route in goats.

### Animal health research in livestock and poultry

**Goats:** John's disease in goats is caused by *Mycobacterium avium* ssp *paratuberculosis*. A IS900 based PCR was developed for rapid identification of this bacteria. For serological diagnosis of John's disease, an ELISA test was also developed. This test is useful for the screening of the goat flocks. Healthy ruminants transiently harbor the human pathogen *Escherichia coli* O157: H7 and other STEC in their gastrointestinal tract. However, prevalence of pathogenic *E. coli* is not clearly understood in goats in Indian sub-continent. Samples were drawn from goat faeces, meat, milk and other sources for isolation of the pathogen, and 38 isolates were biochemically characterized as *E. coli*. Rest of the 138 isolates belonged to *Klebsiella*, *Proteus*, *Enterobacter* and *Micrococcus* spp.

Neonatal diarrhoea is predominantly caused by *E.coli* in young animals of all species, causing 20–40% mortality and other indirect losses. Eight medicinal plants were identified on the basis of their

### Technologies commercialized

- An area specific mineral mixture to increase productivity of bovines i.e. milk yield and body weight in Uttarakhand and Uttar Pradesh
- Development of an indigenous methodology: IVRI cryscope as a field tool for determining optimum time for fertile insemination in animals
- An Asian origin of attenuated homologous vaccine for *Peste des petits ruminants* (PPR)
- Foot and mouth disease vaccine (FMD)



### Patient application filed

- Recombinant chimeric G-protein of rabies virus produced in transgenic plants and synthetic gene for development of vaccine.
- A novel peptide as transfectin reagent for proteins and nucleic acids.
- A process of preparing a bio organo-mineral formulation for the therapy of skin ailment in animals.
- A process to prepare an indigenous drug formulation for the treatment of diarrhoea in animals.
- Development of technology for an area specific mineral mixture to increase productivity of bovines i.e. milk yield and body weight for Delhi region.
- Thresher-cum straw treatment machine.
- A novel immunobiosensor apparatus for rapid diagnosis of FMD in livestock.

antibiogram, antidiarrhoeal property in laboratory rats and clinical trials in kids. A PCR assay from milk and tissues of goat, with primers derived from the omp31 gene sequence of the *Brucella melitensis* was developed. This PCR resulted in the amplification of a 720 bp PCR product. This PCR exhibited a specificity and sensitivity of 100 and 86% respectively.

**Sheep:** Application of planned flock health programme, which primarily involves vaccination against PPR, enterotoxaemia ET and sheep pox, one strategic deworming and one dipping reduced possibility of any outbreak of specific diseases.

**Equines:** Seromonitoring of important equine diseases is being undertaken with special emphasis on indigenous equines to study the magnitude of existing and emerging equine diseases in different states of the country. During the year, active sero-surveillance was conducted in 12 States/ UTs of India, namely Gujarat, Rajasthan, Haryana, Himachal Pradesh, Punjab, Chandigarh, Delhi, Uttar Pradesh, Madhya Pradesh and Manipur. EHV-1 antibodies were detected in 52 of the 1,138 (4.5%) samples, while *Babesia equi* sero-prevalence was detected in 168 of the 955 (17.7%) serum samples tested. None of the samples tested for equine infectious anaemia, African horse sickness, glanders, brucellosis and *Salmonella Abortus equi* infection was detected positive.

The prevalence of equine rotavirus in diarrhoeic foals in organized farms was determined using a monoclonal antibody-based sandwich ELISA developed by the centre. Stool samples were collected during three foaling seasons from diarrhoeic foals from organized farms. Out of 137 samples tested, 46 (33.58%) were positive for rotavirus by ELISA. This indicates that rotavirus-associated diarrhoea is a major problem in foals below 2 months of age.

**Development of EHV-1 vaccine:** Considering the significance of EHV-1 associated abortions in mares, an indigenous

EHV-1 vaccine is being developed and to test the efficacy of the vaccine in experimental ponies, a pilot study was undertaken for comparing the relative pathogenesis of EHV-1 strains isolated from India with particular reference to their ability to induce abortion in pregnant ponies of non-descript breed.

Ponies inoculated with Raj-98 EHV-1 strain showed severe nasal discharge up to 5 days. Profuse vaginal discharge was also seen on day 30 post-infection in mare that gave birth to a weak foal, which was unable to suckle milk of dam and had respiratory distress that died within 24 hr of its birth. Virus could also be isolated/demonstrated from the foetal /dead foal tissues aborted ponies infected with Raj-98 strain.

The study established that Raj-98 strain is more virulent than Hisar-90-7 strain, inducing abortion or foal mortality in 2 out of 4 pregnant ponies. Thus Raj-98 strain of EHV-1 could be used as challenge virus in EHV-1 vaccine trial being undertaken at this Centre.

**Development of improved diagnostics for equine ailments:** During the year, efforts were made to develop recombinant protein-based diagnostic for differentiation of EHV-1 and EHV-4 viruses which are very closely related antigenically. For this glycoprotein G gene from regions of EHV-1 were transformed into competent DH5a *E. coli* cells and expressed. The expressed 41 kDa protein specifically reacted with EHV-1 antibodies. The work on expression of EHV-4 glycoprotein G fragment is under progress. These recombinant proteins will be exploited for their use in diagnostics.

Equine piroplasmiasis caused by *Babesia equi* is a serious problem of major economic importance in equines. To develop improved diagnostics, an ELISA was standardized employing

### Seroprevalence of equine diseases in various states during 2005-06

State	Number of samples tested (positive)	
	EHV-1	<i>B. equi</i>
Rajasthan	180 (5)	180 (42)
Haryana	60 (3)	62 (8)
Delhi	72 (6)	44 (20)
Manipur	40 (2)	40 (6)
Uttar Pradesh	119 (6)	67 (12)
Madhya Pradesh	206 (2)	206 (40)
Gujarat	70	70 (11)
Uttarakhand	232 (23)	232 (28)
Himachal Pradesh	54 (2)	54 (1)
Chandigarh	41 (1)	–
Punjab	64 (2)	–
Total	1,138 (52)	955 (168)





### Technologies patented

- Cold process technology for the preparation of urea molasses mineral block
- Development of technology for an area specific mineral mixture to increase productivity of bovines, i.e. milk yield and body weight in Tarai and hill regions of Uttarakhand

recombinantly expressed merozoite surface protein EMA-2, for detection of *B. equi* specific antibodies. The assay was found specific and no cross-reaction was observed with *B. caballi* and *Trypanosoma evansi* antibodies. In addition, a PCR was standardized using primers to amplify the fragment of 346 bp and 800 bp specific to EMA-1 and EMA-2 genes, respectively. The work on determining the sensitivity and specificity of these assays is under progress.

Diagnosis of surra in equines is difficult when there is low level of parasitemia due to low sensitivity of routinely used parasitological tests. Therefore, a PCR was standardized for sensitive detection of *T. evansi*, using primers designed to amplify the gene of surface glycoprotein Ro Tat 1.2. A gene fragments (761 bp) of Ro Tat 1.2 gene from the Indian strain of *Trypanosoma evansi* was amplified in the PCR. The amplified DNA fragment was cloned and sequenced. The sequence analysis and comparison by BLAST search showed a very high similarity (> 90%) with the sequences of Ro Tat 1.2 gene of other known strain of the *Trypanosmes*.

Rotavirus associated diarrhoea is one of the leading causes of mortality and morbidity in foals below two months of age world over. To detect the equine rotavirus directly from the stool samples, a monoclonal antibody-based sandwich-ELISA was developed by the centre. This assay is very sensitive, specific and detects rotavirus from the stool samples within 4 hr.

**RFLP based genotyping of MHC class II genes in Marwari horses:** Major histocompatibility complex (MHC) genes that code primarily for cell surface glycoprotein play a key role in the regulation of immune response in the animals. The MHC provides a major genetic component of resistance/susceptibility to infectious or autoimmune diseases and regulates the basic immune response in higher animals. In horses, MHC is localized to chromosome 20q14 - q22. There are three functional and expressed MHC class II loci (DP, DQ, DR) and each locus contains class II A and B genes. The polymorphism in the MHC class II gene in 24 Marwari horses was evaluated by RFLP analysis. ELA-DRB3 locus in Marwari horses was homozygous on digestion with *HinfI*, showing two fragments of 241 and 68 bp. On digestion with *RsaI*, the ELA-DRB3 fragment was polymorphic, showing fragments of 238 and 71 and 190 and 119 bp. Similarly, *HaeIII* resolved the fragments of 221 and 88 and 170 and 139 bp. The RFLP results revealed that using above restriction enzymes, the animals could be grouped into different classes.

**Isolation and characterization of seminal plasma proteins of Marwari stallions:** Seminal plasma contains many proteins which may be used as a diagnostic tool/ marker to

### SUCCESS STORY

#### Pestivirus infections of small ruminants in India

Bovine viral diarrhoea virus (BVDV) type 1, BVDV type 2 and border disease virus (BDV) belonging to the genus *Pestivirus* in the family *Flaviviridae* can infect sheep and goats and inflict economic losses in farming. The status of pestivirus infection capable of producing various disease syndromes in Indian sheep and goats was not known earlier. Moreover, border disease is considered exotic to India. Therefore, a study was undertaken to ascertain prevalence of pestiviruses in general and BDV in particular. Blood samples from 651 sheep and 562 goats in 41 flocks of seven Indian states were tested for the presence of pestivirus antibodies and pestivirus antigen by ELISA. Pestivirus antibody positive samples were tested for border disease virus specific antibodies. Prevalence rate of pestivirus antibodies was 26.1% in sheep and 29.1% in goats, and for BDV antibodies it was 7% in sheep and 2.8% in goats. Pestivirus antigen was detected in 11.8% sheep and 8.7% in goats providing serological evidence of infection first time in Indian small ruminants. From sheep 33 pestivirus isolates and from goats 23 isolates were obtained. An RT-PCR assay was standardized for specific detection of border disease virus and a nested PCR was developed for differentiation of BDV, BVDV 1 and BVDV 2 using 5' UTR primers. Another nested PCR was also developed using structural Erns region specific primers to differentiate pestivirus isolates. RT-PCR and nested PCR provided evidence of BVDV 1 and BVDV 2 infection and no BDV infection. The 5' UTR of 16 isolates was amplified by RT-PCR and sequenced. Phylogenetic analysis classified 14 isolates as BVDV 1, and 2 isolates as BVDV 2. BVDV 2 was isolated for the first time in India and identified as 2a subtype having close relationship with North American and European strains. Further genetic analysis of BVDV 2 isolate in Erns and NS5B region proved its subtype besides observation of unique amino acids. Genotyping of BVDV 1 isolates revealed existence of 1b and 1c subtypes whereas earlier we detected BVDV 1b in cattle. BVDV 1c subtype similar to Australian cattle strains was detected for the first time in India and for the first time in sheep and goats. Though clinical signs similar to border disease were noticed in small ruminants and serology indicated BDV infection, genetic characterization established existence of BVDV 1 mostly and BVDV 2 sporadically. Surveillance for BDV, however, needs to be continued.



## SUCCESS STORY

### FMD viral repository crosses the thousand mark

The Project Directorate on FMD maintains one of the largest viral repositories available in the country. The repository was initiated in late seventies. The representative samples of viruses isolated from different field outbreaks are preserved in liquid nitrogen as well as in deep freezers in the repository. The viruses are well characterized antigenically before including in the repository. These isolates have been the raw materials for genetic analysis of the field isolates. Being highly valuable research material, they have helped in understanding the dynamics of viral evolution within the region for assessing the relevance of in-use vaccine strains. Since the virus mutates very fast the circulating field isolates in different times and geographic locations change demanding a constant vigil on the vaccine strain used. On such occasions the viral repository only can act as the saviour in making available an array of viral types to be tested for its usefulness as a vaccine strain for the particular time. This repository also supplies the vaccine and challenge strains of the virus required by different manufacturers of the country. The National repository contained a total of 1,250 (800-O, 235-Asia 1, 200-A, 15-C) field isolates being one of the largest viral repository of the country.

select stallions of high breeding value. A study was initiated to evaluate the effect of gelatin and heparin binding fertility related proteins of stallion seminal plasma on *in vitro* fertilizing potential of spermatozoa. The heparin and gelatin binding proteins were isolated from the stallion seminal plasma. Five heparin binding proteins (17- 83 kDa) and four gelatin binding proteins (18-83 kDa) were identified. The effect of these proteins in fertilizing potential is being evaluated.

**Assessment of technologies:** Internal validation of kit for pregnancy diagnosis: A simple and reliable method of pregnancy diagnosis can help and improve the reproductive efficiency of

mares. This centre has developed an eCG based ELISA for pregnancy diagnosis which is quite economical, sensitive, specific, reliable, easy and animal friendly as compared to conventional rectal palpation technique. This test is effective in diagnosing the pregnancy between day 35 and 120 of gestation. However, this eCG based test is specific only for those mares which have been covered by horse stallion only and not for mares covered by donkey stallion for mule production. Internal validation of this test as well as feed back received from equine owners has indicated 100% sensitivity of this assay.

**Poultry: Standardization of *in ovo* vaccination in broiler**

**chickens:** The eggs injected with *in ovo* vaccines had lower hatchability than the control un-injected eggs. In 15th day vaccinated groups, there was higher number of embryonic death just before piping and had lower hatchability (50.6%) than 18th day vaccinated egg (56.3%). Yolk sac vaccinated eggs (54.2%) had higher hatchability than amniotic sac route (50%). As far as dose of ND vaccination is concerned, increase in vaccine dose decreased the hatchability. At lower dose there was 66.1% hatchability as compared 41.5% in high dose group. 15th day vaccinated birds had significantly higher HI titre (log<sub>2</sub>) value (7.17) for the first four days post-hatch than control (5.32) and 18th day vaccinated group (5.23). From 7th day onward both 15th and 18th day vaccinated birds had significantly higher HI titre value than control birds except on 28 day where 15th day vaccinated group had low HI value than control. When two vaccinated sites (amniotic sac and yolk sac) were compared there was no difference in the titre values among *in ovo* vaccinated and control birds for first four days. From 7th day onward both amniotic and yolk sac vaccinated birds had also significantly higher HI titre value than control birds even at 46 days of age. Control birds maintained HI titre of 4 or above only up to 14th day of age, then there was sharp decrease in titre values. *In ovo* vaccinated birds maintained HI titre of 4 or above even up to 46th day of age.

### Development of novel herbal ectoparasiticide product

Commonly available 30 plants were selected for development of the herbal ectoparasiticide drug. Methanolic extracts for all plants were evaluated for their efficacy on adult ticks and lice of all livestock species. Most potential plants were selected for further studies on the basis of LC<sub>50</sub> values. Brine shrimp lethality test was also performed for simulation studies and further verification of efficacy. Different combinations of plants for synergistic action and concentrations were evaluated for final combination and concentration of the prototypes. Eight prototypes were clinically evaluated in more than 1,000 animals including cattle and buffalo calves, goats, sheep and dogs along with all necessary safety/toxicity trials. On the basis of efficacy and safety studies, one prototype was selected for drug development. Detailed phytochemistry of prototype with HPTLC and GC-MS was completed. This prototype can eliminate 80–100% parasites within two hr without staining and any toxic reaction on skin. Final clinical trials are under way and accordingly patent will be filed. Three different preparations for marketing with brand name "ALQUIT" as spray, shampoo base and water soluble have been finalized. The best product will be available in the market for management of ectoparasitic infection in livestock within six months.



### Diagnosis of highly pathogenic avian influenza and development of killed vaccine

Highly pathogenic avian influenza caused by H5N1 subtype of the virus is a global problem because of its communicability from birds to humans. Till January, 2006 the disease/infection was not reported in India. However, in February, 2006 the disease outbreak occurred in Navapur area of Maharashtra and subsequently in Gujarat and Madhya Pradesh. Using OIE/WHO recommended test procedures the High Security Animal Disease Laboratory (HSADL), Bhopal, could diagnose the disease in a record time of 5 days. The diagnosis was based on virus isolation in chicken embryos, haemagglutination (HA) and haemagglutination inhibition (HI) tests, polymerase chain reaction (PCR) using OIE and WHO primers and real time PCR. On 18th February, 2006 the disease outbreak was notified by the Government of India and communicated to OIE. The Central and State Government machineries in close collaboration with HSADL could control the disease by culling 10.14 lakh birds in the affected areas of 10 KM radius and destruction of 14.75 lakh eggs and 8,600 Metric tonnes of feed and feed ingredients.

During the course of investigation the laboratory could isolate as many as 52 virus isolates, all of which belonged to H5N1 virus. By intravenous pathogenicity test the viruses were found to be highly pathogenic. Representative virus isolates were genetically characterized by DNA sequencing and phylogenetic analysis. The most important observations were that the two

outbreaks in Navapur and Jalgaon of Maharashtra were different and the infections were introduced from two different sources by migratory birds. It was also observed that Navapur virus was a reassortant, the HA gene was from mixed migratory bird (MMB) group and NA gene from Vietnam-Thailand-Malaysia (VTM) group. The virus also contained multiple polybasic amino acids at the cleavage site of the HA gene, which is an indicator for pathogenicity. Presence of amino acid lysine at position 627 of the PB2 gene indicated that the virus can infect mammals including human beings. This is the second chicken isolate in the world, next to Nigerian isolate, which possesses lysine. Genetic analysis of NA gene revealed that Indian virus isolates are sensitive to Oseltamivir (Tamiflu) which is the drug of choice for treatment of avian influenza. Attempts were also made to develop a killed vaccine which could be used for control of the disease in chickens. The killed vaccine using the Navapur isolate of the virus proved to be efficacious and protected more than 90% of the chickens against challenge with virulent field virus. On completion of laboratory trials on the safety and efficacy of the vaccine the laboratory also developed the detailed protocol for vaccine production, which could be transferred to any agency having the required biosafety Level-3 vaccine production facility. The laboratory has now produced more than 80,000 doses of the vaccine for use.



The eggs injected with low doses of vaccine had significantly higher ND titre value than control and other two vaccine doses for the first four days of post hatch period. However, after 4 days all three vaccine doses had higher ND titre value than control birds even up to 46 day of age except at the 28 day where low dose vaccinated group had low HI value than control.

**Mithun:** *Seroprevalence of rotavirus antibody:* The overall seroprevalence of antibodies to rotavirus in mithun was found 75% by using an ELISA test. The seroprevalence of antibodies to rotavirus

among different age groups of mithun was statistically significant. The seroprevalence increased with the increase in age of the animals. Highest seroprevalence was observed in mithuns above 3 years of age with the lowest seroprevalence recorded in mithuns aged between 2–12 months; it was 66% in mithuns of 1–3 years age. The animals with highest degree of (++++) positivity also increased with the increase in age with the highest percentage (54%) of positivity recorded in mithuns above 3 years of age. No statistically significant difference in the seroprevalence was observed between





## LIVESTOCK AND POULTRY IMPROVEMENT AND MANAGEMENT

sexes, however a tendency towards higher seroprevalence was found among females. The influence of management system on the seroprevalence of antibodies to rotavirus in mithun was statistically significant. Highest seroprevalence was observed in mithuns kept in semi-intensive system in comparison to mithuns found in free-range system of management.

**Prevalence of *Cryptosporidium parvum*:** The overall prevalence of *Cryptosporidium parvum* in mithun was found 56% using an ELISA test. The prevalence among different age groups of mithun was statistically significant. The prevalence decreased with the increase in age of the animals. Highest prevalence was observed in mithuns of 1–6 months of age with the lowest prevalence recorded in mithuns above 2 years of age; it was 62% in 7-month to 2-year-old mithuns. The animals with diarrhoea were more likely to be positive (96%) for *C. parvum* than non-diarrhoeic animals (48%). No statistically significant difference in the prevalence was observed between sexes, however a tendency towards higher prevalence was found among males. The influence of management system on the prevalence of *C. parvum* in mithun was statistically significant. Highest prevalence was observed in mithuns kept in semi-intensive system in comparison to mithuns in free-range system of management.

**Bacterial pathogens:** Faecal samples from diarrhoeic calves were collected and analyzed to find out the bacterial pathogens associated with diarrhoea in mithun by standard bacteriological methods. *Escherichia coli* isolates were isolated from faecal samples and these isolates were subjected to antimicrobial drug sensitivity

test. Isolates were susceptible only to kanamycin and streptomycin. In another study all these *E. coli* isolates (obtained from 25 diarrhoeic samples) were subjected to specific etiologic diagnosis of K99<sup>+</sup> *E. coli* by using K99<sup>+</sup> Pilitest. Out of 25 *E. coli* positive samples, only 5 samples were found positive for K99<sup>+</sup> *E. coli*.

FMD is the most common viral diseases (affecting both young and adult mithuns) having economic impact on mithun husbandry, hence mithuns found in their natural habitat (from Chozuba and Porba villages, Phek district) were vaccinated with polyvalent FMD vaccine. Faecal and blood samples from these mithun were collected for investigation of various diseases in free-range mithun.

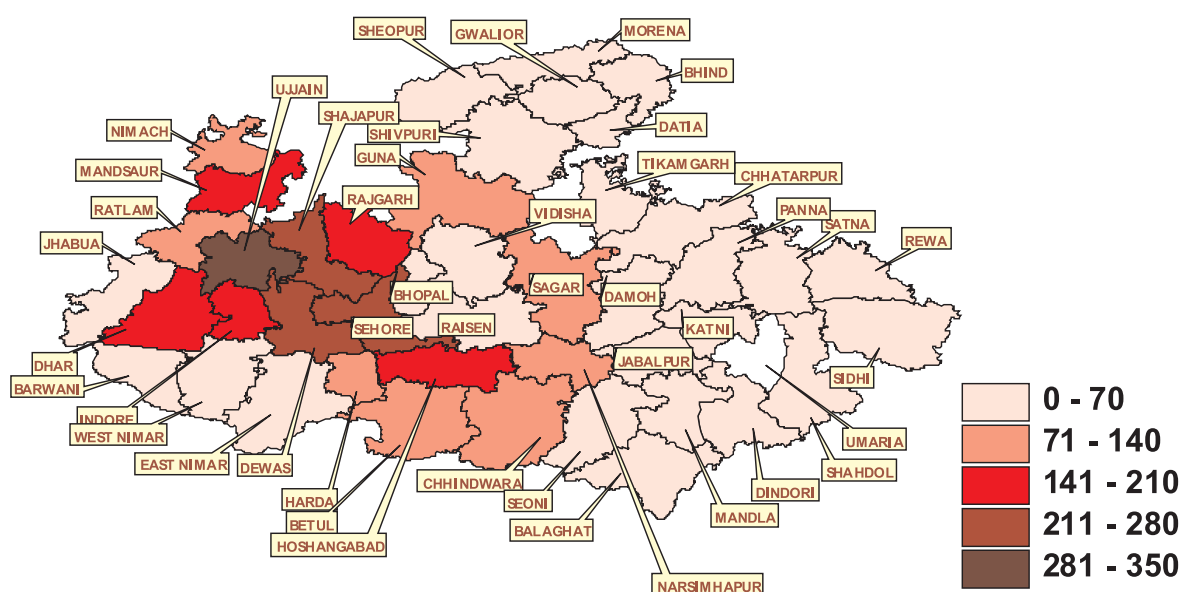
**Yak:** Warble infection in yak due to *Hypoderma lineatum* was recorded. Studies on exploring the mechanism of hepatotoxicity induced by senecio (pyrrolizidine) alkaloids in yak revealed that oxidative damage induced by senecio may be attributed to its toxicity.

## ANIMAL NUTRITION

### Animal feed resources

District-wise information on the feed and livestock resources was collected, assessed and various thematic maps depicting the salient features of distribution of feed and livestock resources were developed for Rajasthan, Madhya Pradesh, West Bengal and Bihar. The diage co-efficients of crop residues of paddy, jowar, groundnut and sugarcane were estimated for Goa, Gujarat, Kerala and Maharashtra.

**Soybean cake availability (000 tonnes) in different districts of Madhya Pradesh**





- Driage coefficients of crop residues estimated
- WNG-12, an anaerobic fungi, improved nutritive value of wheat straw
- Clove oil found most effective against *Aspergillus*, *Penicillium* and *Fusarium*
- Anifeed in ration of lactating crossbred cows improved milk yield by 10.16%
- Vitamin E-selenium injections improved udder health of cows
- Supplementation of 2% activated charcoal in the diet of lactating cattle reduced residual pesticide in milk
- *Pleurotus ostreatus* fungi proved more efficient in lignin breakdown
- Extracts of *Allium sativum* reduced methanogenesis
- Zinc-sulphate treated soybean cake enhanced protein utilization
- Byproducts based complete feed was prepared for intensive goat production
- Low cost animal grade wheat could be safely used in sheep diet
- Apricot seed cake could be used up to 10% in rabbit ration
- Complete feed blocks improved milk yield in lactating camels
- Yeast enzymes supplementation improved digestibility of pig feeds
- Mithuns fed on feed blocks showed improved performance
- Zinc (Zn) and manganese (Mn) combination enhance immune response in poultry
- Dietary incorporation of sun-dried cage litter up to 10% in layer bird ration did not affect egg production, egg weight and egg shell quality
- Increase in vitamin E in the broiler diet improved their immune competence
- Earthworm meal found as an alternative ideal animal protein source for poultry
- Formic acid (1%), propionic acid (1%), lactic acid (1%) and fumeric acid (0.5%) in quail diet proved suitable dietary alternate for antibiotic feed supplement
- Efforts are being made to produce designer egg through nutritional manipulation of diet

## Cattle

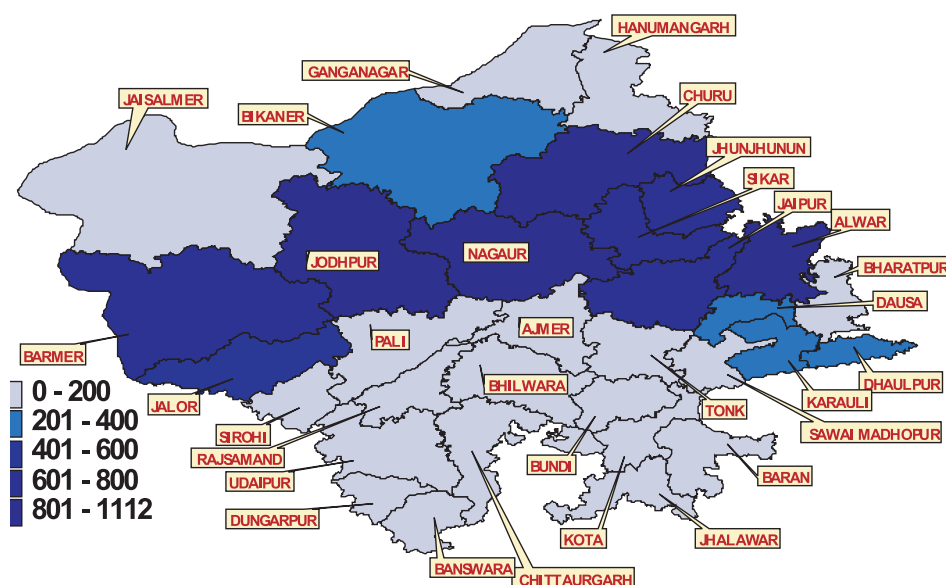
**Microbial protein:** The protein needs of ruminants in terms of rumen degradable nitrogen (RDN)/protein must be supplied to meet the nitrogen requirement of rumen micro-organisms to optimize microbial growth and efficiency. This is essential for increasing the fermentation of poor quality crop residues.

*In-vitro* studies were carried out with individual as well as different combination of supplements to supply different quantities of fermentative nitrogen to optimize RDN by enhancing the

fermentation of ragi straw. Low ammonia-N and slightly higher/comparable TVFA levels were observed at 15 g level of supplementation for groundnut cake, soybean cake, cotton seed cake, 22 g for sunflower cake, and at 30 g RDN for wheat bran.

**Rumen fungi:** *Anaeromyces*, *Orpinomyces*, *Caecomyces* and *Piromyces* fungi were isolated and characterized from different faecal samples of cattle, buffalo, goat sheep and wild animals. WNG-12 was found to be a promising rumen anaerobic fungi to act as a probiotic to improve nutritive value of wheat straw.

**Availability of Bajra straw (000 tonnes) in different districts of Rajasthan**





### Amelioration of mycotoxin

Studies on preventing mycotoxin production in feeds using plant derived compounds showed that clove oil is the most effective against *Aspergillus*, *Penicillium* and *Fusarium* species of fungi, followed by turmeric powder, garlic extract, neem oil, asafetida powder, pepper powder and ginger extract.

Anti – fungal activity of plant extracts

Plant extract	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Fusarium</i>
Clove oil	++++	++++	++++
Garlic extract	++	+	+
Eucalyptus oil	+	+	++
Jerra powder	++	–	++
Karanj oil	++	+	++
Neem oil	++	++++	+
Asafetida powder	++	++++	+++
Cardamom powder	+	–	+
Turmeric powder	++	++++	+
Pepper powder	+	++++	+
Ginger extract	+	++	++
Onion extract	–	–	–
Coriander extract	+	–	–
Thulasi leaves extract	+	–	–

\*Extent anti – fungal activity: high 80-100% +++++; good 60-80% ++++; medium 40-60% ++; low 20-40% +; poor 10-20% -; nil < 10 –

### ICAR-ACIAR Australia Collaborative Project—Increasing productivity of cattle with rumen fungal treatments:

Isolation and purification of anaerobic fungi from rumen and faeces of cattle and buffaloes were completed. Out of the six species of piromyces, only two species were isolated of which *Piromyces communis* was predominant in all samples studied. The occurrence of anaerobic gut fungi *Cyllamyces genera* was reported for the first time in Indian cattle.

**Bio-availability of micronutrients:** Feeding of concentrate mixture having more wheat bran/groundnut cake as practiced under field conditions contributed to higher P intake, resulting in its higher excretion in dung and also affecting the utilization of other nutrients. Decreasing the P level in the concentrate mixture through the addition of other energy source like maize and corresponding increase in Ca level through inclusion of calcium carbonate was advantageous.

**Effect of management:** Higher concentrate allowances in the ration along with effective evaporative cooling system was the best

strategy to maximize production of crossbred cows under hot environmental conditions. During winter the extra climatic protection provided to crossbred cows gave no added advantage in terms of production performance with particular reference to moderate producing cows especially when ample green fodder (berseem) was available.

**Bypass fat supplementation:** In medium or high yielding crossbred cows bypass fat @ 300 g/day/animal significantly increased the milk yield and FCM yield up to 15.61 and 24.01%, respectively. Rumen protected DL-methionine (50 : 50 with formaldehyde (HCHO) treated maize flour) was similar to the formaldehyde protected mustard cake in terms of milk yield and its composition.

**Feed supplements:** Dietary supplementation of anifeed to lactating crossbred cows as per the recommended dose, increased milk yield by 10.16%.

A balanced concentrate mixture was formulated to encounter both protein and energy deficiencies in cattle of old Alluvial Zone of eastern region of the country. It contained local feed ingredients, viz. mustard/linseed cake, rice bran, rice grit, lime and salt in the ratio 38 : 30 : 30 : 1 : 1. Monensin supplementation provided nutritional and metabolic advantage to heifers around puberty. Vitamin E or vitamin E-selenium injections improved the udder health of cows.

**Pesticides and nutrient utilization:** Adding chloropyriphos and methyl-parathion at 100 ppm showed no effect on dry matter and organic matter solubility of paddy and ragi straw but there were differences in proximate composition. The combination of these two pesticides resulted in lower dry matter solubility in ragi straw. Supplementation of 2% activated charcoal in the diet of lactating cattle reduced the level of residual pesticides in milk.

**Bio-energetic and environmental studies:** *Chaffed feeding:* Feeding of chaffed ragi straw (finger millet–*Elusine corcana*) reduced energy expenditure and CO<sub>2</sub> emission by animals and activity of most of the cellulolytic and hemicellulolytic enzymes increase in the rumen liquor.

*Solid state fermentation:* *Pleurotus ostreatus* fungi proved more efficient in lignin breakdown as compared to *P. sajorcaju*. *Voriella volvoraceae*, *Phanerochaete chrysosporium*, and *T. hirsuta* did not prove to be efficient in the lignin breakdown of ragi straw. High activities of CM cellulase, xylanase, acetylesterase,

### Fodder production

Pre-emergence application of atrazine @ 1 kg/ha level gave a season long effective control of weeds resulting in maximum grain yield (56.26 q/ha), stover yield (84.45 q/ha) and biomass yield (140.71 q/ha).





protease,  $\beta$ -glucosidase and micro crystalline cellulase were recorded upon fermentation with all these five fungi. CM cellulase isolated from *Pleurotus* gave a yield of 26% with a 2.4-fold purification. The properties of the partially purified enzyme showed it to be optimally active at pH 6.8 and the enzyme was stable over a wide pH ranging between 5 and 9.

**Utilization and treatment of conventional and non-conventional feeds:** Conversion of castorbean meal into wholesome protein substitute was successfully attempted after processing with sea salt, lime, and by water washing. Extracts of *Allium sativum* and *Populus deltoids* with solvents ethanol and methanol, respectively, induced significant reduction in methanogenesis without affecting *in vitro* digestibility. Bioactive plant secondary compounds (tannins and saponins) derived from tannin-rich tree forages, *Ficus infectoria*, *Ficus roxburghii* and *Quercus incana* were evaluated as natural feed additives for increasing rumen fermentation efficiency. The additive effect of CTs, tannic acid (HT) and triterpenoid saponins (S), and their combinations along with a protein rich locally available forage mulberry (*Morus alba*) were evaluated by *in vitro* gas production technique. Addition of CT led to decreased C2: C3 ratio and decreased MPS. Addition of HT+S yielded the maximum response. The EMPS increased with CT+HT; but there was mostly a decreased response with S. True degradability and partition factor showed better response with CT and S. The supplementation of tannins @ 3% in diet benefited growing animals. However, beyond 3% tannins

### Updating nutrient requirements and their bioavailability

Requirements, bioavailability and inter-relationships of different dietary nutrients such as metabolizable energy, amino acids (lysine, methionine, threonine, and tryptophan), minerals (zinc, manganese, and chromium) and vitamins E for different classes and age groups of various avian species of economic importance under tropical conditions were established. The dietary protein concentration could be reduced by 1.6% in starter and finisher broiler diets through supplementation of L-threonine without affecting performance, immunoresponsiveness and feed cost of production. Similarly, the protein could be reduced from 25.46–23.21% in diets of growing Japanese quails through L-threonine supplementation to match the requirements following ideal amino acid concept. The revised and updated version of computer software "MAKEFEED POULTRY" for feed formulation was also developed.

level in diet required treatment with either PEG or calcium hydroxide. The leaves of *eupatorium* plant (*Eupatorium adenoborum*), which are rich in crude protein (nearly 25%) were found promising as an unconventional feed resource.

### Buffalo

Development of economic rations is very essential for various categories of buffaloes. Costly cakes like groundnut cake (GNC) could be replaced up to 50% by expeller pressed mustard cake, and

### Hay making under polyhouse

Polyhouse with natural ventilation was used for hay making to achieve faster drying rate. The experiment was conducted to observe the drying rate of *anjana* grass (*Cenchrus ciliaris*) in the polyhouse under ambient conditions. The drying rates were higher inside the polyhouse than ambient solar drying. Use of polyhouse for hay making prevented charring by direct sunlight and leaching of nutrients by unwanted rain during hay making.



Panoramic view of polyhouse



Drying inside polyhouse



up to 100% by soyabean meal or sunflower cake in the ration of growing and lactating buffaloes without any adverse effect on growth or milk production. Most economical ration with a targeted body weight gain of 500 g/head/day was all forage diet, or a diet containing ammoniated wheat straw+ available green forage and 30% barley. Under the circumstances when cereals are to be spared for human population, grainless diets can be fed to growing buffaloes with a body weight gain of 500 g/head/day. Protein utilization enhanced in buffaloes fed on zinc sulphate treated soybean cake possibly through reduced ammonia concentration and protozoa number in the rumen of buffaloes.

### Goat

**Sustainable goat production on semi arid pastures:** Goats are mainly reared in semi arid region, which is deficient in available herbage to the grazing animals. Models, viz. natural pasture, *Cenchrus ciliaris* pasture and *Tephrosia purpurea* + *Cenchrus ciliaris* pasture were compared by introducing growing six kids (3 male and 3 female) of 4–5 months age in each model.



The initial body weight of the kids in the natural system was  $8.65 \pm 0.67$  kg and in pure *Cenchrus ciliaris* pasture  $8.65 \pm 0.87$  kg. Mixed pasture of *Tephrosia purpurea* and *Cenchrus ciliaris* had kids of  $8.77 \pm 0.85$  kg weight. After 150 days of grazing, average daily gain was  $35.11 \pm 5.44$  g in natural pasture,  $39.88 \pm 7.00$  g in *Cenchrus ciliaris* + *Tephrosia purpurea* pasture and  $39.86 \pm 6.00$  g in *Cenchrus* pasture.

**Byproduct based complete feed:** A growth trial using post weaned kids was conducted on the ration based on agricultural and agro-industrial byproducts in Barbari, Jamunapari and Marwari goats. Barbari breed responded better with this type of by-products based ration under intensive system.

### Sheep

**Improvement of feed resources and nutrient utilization in raising animal production:** Activities of Cu and Zn-

dependent enzymes (Cu-Zn superoxide dismutase, ceruloplasmin and alkaline phosphatase) responded positively on dietary levels of Cu and Zn, and increased on increased supplementation of Cu and Zn in the diet. Plasma Cu and Zn levels also increased on increased supplementation of dietary Cu and Zn. An increase of 16–22% in Cu and 4–8% in Zn was observed on 50% and 100% more supplementation of Cu and Zn over the unsupplemented control diet. Body weight gain was higher in 100% more Cu and Zn supplemented animals than the animals of other groups. Cu/Zn-SOD enzyme activity positively correlated with dietary levels of Cu and Zn and ceruloplasmin activity with plasma Cu. Thus enzymes Cu/Zn-SOD may be used as an indicator or biochemical marker for assessing both Cu and Zn status and ceruloplasmin for Cu status of sheep. Bioavailability of Cu and Zn was better in sheep fed organic sources of Cu and Zn in the diet. Gut absorption of Cu and Zn was higher and faecal excretion of Cu and Zn was lower in organic compared to inorganic group suggesting better utilization/storage of these minerals. Relative bioavailability based on multiple linear regressions was about 141 and 122% in organic Cu and Zn supplemented group. The availability of Cu and Zn in wool and tissues especially in the liver was higher in sheep fed organic sources of Cu and Zn compared to inorganic sources.

The utilization of different types of pastures and silvi-pastures for sheep production was monitored. The herbage of grazing lands in these regions contains organic matter (OM) 78% with a crude protein (CP) of 10%. Cell wall carbohydrates (CWC) in the herbages were about 55 to 58% with 23.04% of cellulose and 3.91% lignin. The carrying capacity of grazing lands was only 0.41 adult cattle units (ACU)/ha but the stocking density was 11 ACU/ha. The diet samples of pregnant ewes contained 83.39% OM, 13.50% CP, 52.30% NDE, 28.63% ADE, 28.63% cellulose and 6.69% lignin. DM, DCP and ME intakes on metabolic body size in supplemented and non-supplemented groups were 43.65 and 38.44 g/day, 2.82 and 1.44 g/day and 0.57 and 0.50 MJ/day respectively. Under intensive system lambs for mutton may be fed on low cost animal feed grade wheat replacing up to 75% costly energy supplement like maize to economize cost of production. Maize could also be replaced even 100% with animal feed grade damaged wheat without compromising production performance.

### Rabbit

Apricot seed cake, a byproduct, is generally not fed to livestock due to presence of cyanogens. Apricot seed cake after water treatment may substitute groundnut expeller up to 10% in rabbit ration as a non-conventional feedstuff.

### Camel

**Complete feed blocks:** Feeding and nutritional trials of





## SUCCESS STORY

### An area specific mineral mixture to increase productivity of animals

Farmers generally are not aware of the importance of minerals in the balanced diet/nutrition of animals particularly for animals in production and the need for supplementation through designer mineral mixtures. Most of the problems associated with minerals are area specific, as distribution of essential mineral in feeds, fodder and soil are different in different regions. Area specific mineral mixtures were developed based on the soil-plant-animal status of various dietary essential minerals, and the efficacy was verified through feeding trials on growing and lactating cattle of Uttranchal and Uttar Pradesh.

The designer mineral mixture recommended for these two northern states include Ca, P, Mn, Fe, Cu, Co, Zn, I and Se at optimal concentrations to balance the deficiency of these minerals in diets of livestock. These mixtures were fortified with essential vitamins A, D, and E and a probiotic brewer's yeast. Fortification with essential vitamins and brewer's yeast enhanced the



Haifer with multiminer deficiency suffering from delayed puberty

usefulness of these products. The production and reproductive performance of cattle and buffaloes improved and also their disease resistance ability. The quality of milk also improved with a concurrent increase in lactation length up to 300 in comparison to 180 days to 240 days in animals, which were not given area specific mineral mixture. This lactation length resulted into higher economic returns in terms of approximately 90–300 kg. This gave additional income of Rs 1,530 to 5,100, while the improvement in breeding efficiency significantly decreased the dry period to 60 to 120 days from 180 to 300 days.

Significant acceleration in growth rate was also observed wherein cow and buffalo heifers attained puberty at 18 to 24 months and 24 to 30 months age, as against approximately 30 to 36 months and 36 to 42 months, respectively. The designer mineral mixtures with a positive breeding performance contributed to higher productivity and economic returns from the dairy animals.

complete feed blocks using various combinations of feed materials with minimum proportion of concentrates were conducted on growing camel calves, draught, lactating and breeding male camels. The body weight gain was higher in growing camels and lactating camels given complete feed block than those given only single fodder. The draught camels had better health, worked for longer period and generated more income than when fed only on *moth* chara or *guar phalgat*. The lactating camels produced

significantly higher milk per day when given complete feed blocks than camels fed on sole *moth* fodder, which was also economical at the same time. The stud camels generally loose about 16% body weight during breeding season (December–March). The body weight loss was less when fed complete feed blocks as compared to those given only *moth* fodder. The intake of various nutrients in the form of energy, protein, minerals was also uniform so the camels are saved from the gastric problems of acidosis/alkalosis.







### Pig

Yeast culture (*Saccharomyces cerevisiae*) extract at 1% level in feed proved efficient in increasing average daily gain (ADG) in growing pigs compared to finishers. Yeast enzymes improves digestibility of feeds and thus help in attaining early growth.

### Yak

Yaks under semi-range system of rearing had higher growth rate, attained mature body weight earlier (4 years vs 5-6 years), reduced age at first calving, better productive and reproductive performances compared to yaks reared in field conditions.

### Mithun

**Tree leaves based total mixed ration:** Tree leaves, herbs and shrubs are available in plenty for feeding of mithun in north eastern states. Some of the fodder tree leaves and shrubs are excellent in terms of protein, minerals and digestibility of different nutrients for feeding of mithun.

Perennial tree leaves of *Ficus hirta* and herb like *Borreria hirticulata* could be incorporated up to 30% of total mixed ration without adverse effects on nutrient utilization and growth performance. *Borreria hirticulata* based ration showed marginal

showed promising results for better growth rate, nutrient utilization and nitrogen retention.

### Poultry

**Trace mineral interaction:** Retention of Mn and Zn in bone and liver increased with the level of their inclusion in broiler feed. Zn retention in bone was improved by Mn supplementation, but not the vice-versa. Mn levels depressed Cu retention in bone. Mn and Zn at higher levels in feed adversely affected Cu retention in liver. The combination of Zn and Mn at 160 and 120 ppm enhanced the immune response and the response was more conspicuous with Zn than that on Mn. Source of Zn did not affect the body weight and feed conversion efficiency. Organic Zn was useful in maintaining low leg scores and better antibody titres compared to inorganic salt. Inorganic Zn produced better tibia weight and strength compared to organic Zn. Organic form of the mineral was more efficient in bringing out the desired effect in respect of bone traits, carcass parameters or immune parameters.

**Optimum energy and protein requirement:** The energy and protein requirement of female parent line of Vanaraja during juvenile stage (0–6 weeks) was 2,650 kcal ME/kg diet and 20% crude protein.



Temichiede (*Ficus hirta*) Herb



Phegwe (*Thysanotus agrostis*)



Khuki: (*Osbeckia* sp.) Shrub



Medzei (*Spondias pinnata*) tree

advantage over *Ficus hirta* based in terms of nutrients efficiency and growth. The rumen fermentation pattern showed an established trend in both the treatments but *B. hirticulata* fed animals proved to be more stable as is evident from the efficiency of nutrient utilization and growth in animals fed on this particular diet.

**Feed blocks:** The feed blocks were prepared at the NRC on Mithun, Nagaland, by incorporating urea treated paddy straw and concentrate mixture at 70 : 30 ratio for the first time for these animals. Feeding of these feed blocks



**Recycling of sun dried cage layer manure as feed for laying chicken:** Recycling of poultry excreta as feed may help alleviating pollution problems, decreasing feed cost and increasing supplies of available nitrogen and essential mineral sources. Dietary incorporation of SDCLM (sun dried cage layer manure) did not affect egg production, egg weight and egg shell quality indicating that it could be incorporated up to 10% in the diet of layers without affecting their performance.

**Performance on low nutrient diets:** Gramapriya birds need initial brooding and other management care till they attain the desired body size prior to leaving them in the free range scavenging system. Body weight gain increased with increase in energy up to



2,500 kcal ME/kg, and feed intake decreased progressively with energy concentration in diet. The humoral immunity was not affected by the energy concentration in diet. Lymphocyte proliferation increased with increase in energy level in diet. The protein level in diet did not influence body weight gain, while feed efficiency improved with increased protein in diet. Gramapriya birds needed less protein (18%) and energy (2,500 kcal ME/kg diet) during their juvenile phase of growth.

**Effects of vitamin E on broiler chicks:** The source of oil (sunflower, palm oil, safflower oil) or the level of vitamin E in diet did not influence chick performance. The activity of lipid peroxidase and H:L ratio, which indicate stress decreased progressively with increase in concentration of vitamin E in diet. Immune competence as assessed in terms of CBH response and lymphocyte proliferation ratio increased with increase in level of vitamin E.

**Lysolecithin from rice bran:** Body weight and feed conversion efficiency were higher and feed consumption was lower in the birds fed higher levels of lysolecithin, viz. 0.8 and 1.6% in diet. The slaughter variables, serum lipid profile, liver and muscle fat content and the immune response were not affected. When LL was dissolved in rice bran oil and added to broiler chicken diet at graded levels (0.025–1.6%), no effect was observed on performance and the other parameters.

**Protein and critical amino acids:** Diet with marginally low (–10%) level of protein and TSA (total sulphur acid) gave the highest body weight. Similar growth was seen in the group fed low (–10%) level of protein but with normal amino acids. Dietary content of protein and amino acids could be optimized for better performance of *Krishibro* chicks.

**Utilization of certain alternate energy and protein:** Several alternate feed ingredients such as *guar* meal, quality protein maize, earthworm (*Eisena fatida*) meal and transgenic crops like Bt cottonseed meal and Bt brinjal meal were evaluated for nutritional quality and safe/effective inclusion levels for incorporation in poultry rations. The feeding value of quality protein maize was comparable to commercial maize for broiler chicken and could be substituted up to 100% level for commercial maize in maize-soybean meal based diet. Earthworm (*Eisena fatida*) meal, processed after 6 hr of starvation contained good amount of lysine, methionine, threonine and arginine. Therefore, the meal would serve as an alternative ideal animal protein source for poultry and other non-ruminant species. Low gossypol transgenic cottonseed meal could be included @ 10% in broiler diets safely.

**Augmenting digestibility/nutritive value of the feedstuffs:** Dietary supplementation of 1% each of formic and propionic acid and 0.5% fumeric acid was effective for optimum growth performance, nutrient retention and to control intestinal

colonization and exclusion of harmful microbes in broiler quails, and could be used as suitable dietary alternate for antibiotic feed supplement.

In laying quails 1% formic acid and/or 1% propionic acid was effective for optimum egg production performances, immune response and to control colonization of harmful microbes in the ceaca. Use of 1% each of formic, propionic, lactic and 0.5% fumeric acid in feed resulted in promising effects on decontamination and prevention of recontamination of feed during storage up to 40 days. Feeding live culture of *Lactobacillus acidophilus* or *S. cerevisiae* @ 100 g/kg improved nutrient utilization and immune response and reduced serum cholesterol in quail broilers. In laying quails, *L. acidophilus* or *Saccromycis cerevisiae* cultures in feed improved egg mass and nutrient utilization, similar to antibiotics supplementation. Dietary addition of MOS and FOS to laying quail feed @ 1 g/kg diet enhanced the nutrient utilization and immune response of quail layers. Efforts were also made to produce designer egg through nutritional manipulation of diet.

**Exploring implications of incriminating substances in diet and their amelioration:** Combination of aflatoxin B1 (AF) and ochratoxin (OA), untreated cottonseed meal (UCSM) and AF and treated cottonseed meal (TCSM) and OA resulted in synergistic toxic effect in broilers. The interaction of two mycotoxins (AF and OA) on liver lipid and faecal fat content was characterized as antagonistic. The cotton seed meal because of high free gossypol (0.8%) caused toxicity in broilers, which could be averted by solvent extraction of meal and addition of iron. Combination of AF or OA with UCSM exerted synergistic toxic effect.

**Hatchability and growth performance:** Hatchability was apparently higher in all linoleic acid and 0.25 IU vitamin E injected group than the sham control. Though there was no difference in chick weight and egg weight, but their ratio was higher in 0.25 IU vitamin E and 25 mg linoleic acid group than un-injected control. Body weight at 28 days of age was higher in all vitamin E and 75 mg linoleic acid groups than sham and un-injected control. At 49 days of age 0.25 and 0.50 IU vitamin E and 75 mg linoleic acid groups had 63.6–109.8 g higher body weight than un-injected control. However, FCR was not affected by *in ovo* injections of vitamin E and linoleic acid.

## ANIMAL PHYSIOLOGY AND REPRODUCTION

### Cattle

**Effect of nutritional and managerial interventions:** Crossbred cattle diagnosed for anoestrus, repeat breeding, silent heat, late puberty etc. were supplemented with area specific mineral mixture under Institute Village Linkage Program (IVLP), and 55% of supplemented cows conceived in 60–90 days of supplementation.



- Time lag between heat expression and conception in cows could be reduced by mineral mixture supplementation
- Pre-partum milking training in milk parlour improved milking temperament of cows
- Isolation and partial purification of buFSH, buLH and buPRL were completed
- Efficiency of ovsynch treatment was improved for estrus synchronization/ovulation induction in anestrus buffalo heifer during summer
- Embryos of transferable stage were successfully produced using oocytes derived from *in-vitro* grown prenatal follicles—apparently first report in buffaloes
- Difference in protein profile of seminal plasma and sperm membrane may help developing molecular marker for assessing buffalo bull fertility
- Kids of 5.5 to 7.0 months of age showed fully developed thermoregulatory mechanisms
- Effect of ram was more pronounced in multiparous ewes compared to nulliparous ewes
- Peripheral blood GH levels influenced the temperament in mithuns
- Tris-egg yolk diluents could preserve mithun semen at refrigeration temperature
- Modulation of prolactin improved egg production in poultry
- Manipulation of gut melatonin improved broiler performance
- Progesterone and estrogen analogues administration improved egg production in birds
- Non-hormonal preparations enhanced egg production by 15 egg/bird

### Pre-partum milking training in milk parlour

In Karan Fries heifers, one week of pre-partum training in the milking parlour was adequate in improving the productive performance and milking temperament of these animals. In Sahiwal and Murrah heifers more than two weeks of pre-partum training was required to fully adapt to milking routine.

semen of different age groups were nonsignificant. Out of the total abnormalities (33.54%), the head and tail abnormalities were predominant (13.77 and 13.64%, respectively). The free heads in the semen decreased with the increase of age of the bulls. Among the various abnormalities of mid piece (6.12%) looped mid piece contributed the maximum proportion (2.56%). A large proportion of the spermatozoa showing tail abnormality had proximal droplet followed by strongly coiled (Dag defect) and tail looped on head indicating production of immature sperms and some defects in spermatogenesis.

The semen quality parameters were the best in egg yolk tris extender followed by soya milk based and milk based extenders. Microbiological aspects of semen extended in various extenders at room temperature revealed that the total plate count was the lowest in soya milk based extender followed by egg yolk tris and milk diluent.

### Buffalo

**Isolation and purification of buffalo hormones:** Isolation and partial purification of buFSH, buLH and buPRL were completed. Antisera for progesterone and oestradiol-17 $\beta$  were characterized with binding studies and are being used in radioimmunoassay of progesterone and oestradiol-17 $\beta$ .

**Reproductive efficiency:** During cyclicity, the plasma prolactin concentrations were 40- to 70-times higher in buffalo heifers during summer than that during winter. High prolactin levels in buffalo heifers could contribute to poor fertility by lowering gonadal hormone production in summer. The ovsynch protocol for estrus induction/synchronization could be a potential tool for improvement of fertility in repeat breeding buffaloes even during extreme summer. Norprolac induced prolactin inhibition improved the efficiency of ovsynch treatment for estrus synchronization/ovulation induction in anestrus buffalo heifers during summer.

**Embryo resource generation:** Techniques for isolation and *in vitro* culture of preantral follicles, collection of immature oocytes from antral follicles through ultrasound guided oocyte pick up (OPU) method from live buffaloes and ovaries of slaughtered buffaloes, and maturation and fertilization, were standardized.

Time lag of 150–210 days duration between heat expression and conception, resulted in production loss, which could be reduced by more than 50% if mineral mixture supplementation is initiated earlier.

**Semen production:** The semen production performance of Frieswal bulls was estimated using the semen quality parameters—semen volume ( $43.35 \pm 0.38\%$ ), mass motility ( $2.16 \pm 0.02$  on 0–5 scale), sperm concentration/ml ( $1,175.19 \pm 11.46 \times 10^6/\text{ml}$ ), % motile sperm after dilution ( $43.35 \pm 0.38\%$ ), and per cent motility of sperm after freezing ( $32.76 \pm 0.47\%$ ). The post-thaw motility ( $36.45 \pm 0.78\%$  and  $32.65 \pm 0.91\%$ ) in summer and rainy season were significantly better than that in winter ( $29.99 \pm 0.74\%$ ). Semen ejaculates with a low sperm concentration (up to 500 million/ml) also had significantly low mass motility ( $1.21 \pm 0.04\%$ ) and per cent progressive motility ( $24.41 \pm 1.01\%$ ) compared to ejaculates having more than 500 million sperm/ml.

The overall measures of length, width and depth of left and right testis indicated that scrotal circumference increased with the advancement of age, but it does not meet the minimum standard of 34 cm at the age of 24 months. Sperm abnormalities in the





Embryos of transferable stage were successfully produced using oocytes derived from the *in vitro* grown preantral follicles, and this apparently is the first such report in buffaloes. Vittrification method was standardized and successfully used for buffalo embryo cryopreservation. Oocytes collected from live non-descriptive buffaloes using OPU technique were fertilized to produce transferable embryos. Brilliant cresyl blue staining technique for better selection of oocytes for *in vitro* embryo production was standardized for buffaloes.

**Intra uterine protein profile:** Embryo survival depends on the uterine secretions till its final attachment with uterine wall. Non-cycling animals revealed lower total superoxide dismutase (SOD) compared to cycling animals. Three molecular weight forms of SOD were detected in uterine fluid of pregnant, non-pregnant, anoestrus, infected and non-infected tract. These protein samples were subjected to separation in gradient pH gel and showed differences in protein bands. Pregnant samples contained bands at pH value 5.25, 5.6, 5.9, 6.6, 6.7, 7.0, 7.2, which were not observed in non-pregnant secretion. Similarly three bands at 5.8, 7.4 and 7.5 pH were observed in non-pregnant secretion, which were not found in pregnant animal secretion. Comparison of protein bands revealed at least 8 separate bands in allantoic fluid not similar to amniotic fluid bands. Differences in protein pattern were revealed more prominently by two dimensional electrophoresis analyses.

**Male reproduction:** Computer assisted semen analysis (CASA) is being used to study different aspects of semen quality. Using these facilities guidelines for different buffalo bull semen parameters are being generated. The average, motility, post-thaw motility, and viability for limited number of samples were 43, 17 and 78%. To establish most suitable glycerolization protocol sperm viability and acrosomal integrity at two different temperatures were recorded. SGOT and AKP enzymatic activities were not significantly different in two glycerolization protocols. However higher activity of these two enzymes in neat seminal plasma, suggests damage during cryopreservation process. The backward motility was not found associated with changes in the seminal plasma enzyme profile.

**Semen fertility:** Mitochondrial membrane potential by JC 1 staining, and plasmalemma integrity measurement by Hypoosmotic swelling Giemsa were observed as important determinants of buffalo sperm quality influencing cleavage rate and thus fertility. The differences in protein profiles between bulls were observed in seminal plasma and sperm membrane heparin binding profile (approximately 30 kDa) suggesting the importance of this as molecular marker in assessing buffalo bull fertility.

## Goat

***In vitro* generation and transfer of embryos:** The average

## SUCCESS STORY

### Production of *In-vitro* fertilized-embryo transfer kid in goat

Goat oocytes were collected by follicle puncture method from the ovaries brought from an abattoir. Recovered oocytes were subsequently matured, fertilized and developed to 8-cell stage as per standard protocol. *In-vitro* produced eight-cell embryos were transferred into two surrogate goats of non-descript breed through surgical technique. Out of the two, one goat got pregnant and kidded a healthy female kid weighing 3.15 kg after 146 days of gestation. This is the first IVF-ET kid born at this institute and second in India.



recovery of oocytes using follicle puncture and aspiration was 2.07 and 1.26/ovary. In TC 199 supplemented with 20% EGS, 20% NCS, 20% CFF, 10% EGS, 10% EGS + FSH + LH and 10% NCS + FSH + LH + E<sub>2</sub> the maturation rates of goat oocytes were 77.76, 88.17, 89.32, 71–69, 75.68 and 89.63, respectively, and cleavage rates of *in-vitro* matured goat oocytes were 50.81, 41.54, 42.01, 12.92, 36.31 and 43.16%, respectively. Higher number of embryos were developed to morula stage on co-culture of fertilized oocytes with OEC than that on GCM.

Out of 5 frozen thawed embryos, 3 were normal and the two embryos of 4-cell stage developed to next stage after 24 h of incubation at 38.5°C in an atmosphere of 5% CO<sub>2</sub> in humidified air.

### Synchronization and superovulation of anoestrus goats:

Crestar ear implant (1.5 mg norgestomet s/c for 9 days) was effectively used for oestrus synchronization in donor and recipient goats during non-breeding season. The response of PMSG + FSH-P combination was better than FSH-P alone in terms of ovulatory response and embryo recovery.

**Thermo-adaptability in goat:** Thermoregulatory mechanism in kids are fully developed at about 5.5 to 7.0 months of age. The comfortable zone in adult goats lies between 9.0°–38.5°C. Although the buck can regulate their deep body temperature at about the constant level but they use cutaneous evaporative



## LIVESTOCK AND POULTRY IMPROVEMENT AND MANAGEMENT

mechanism in hot-dry period or respiratory evaporative mechanism in hot-humid period to loose extra body heat. Thermal environment had distinct effect on feed and water intake and blood biochemical profile of goats.

### Sheep

**Fertility studies:** A fertility trial was conducted in 100 cycling Bharat Merino ewes for one cycle during autumn using frozen-thawed semen through transcervical artificial insemination. Ultrasonography revealed that 19 ewes were pregnant.

**Reproductive behavior:** Reintroduction of rams to ewes after isolation of 90 days during non-breeding season for 30 days resulted in induction of estrus. Similarly, reintroduction of rams to ewes following 30 days isolation resulted in synchronization of estrus in the next 30 days. The response of multiparous ewes to ram effect was much more pronounced than that in nulliparous ewes. When the ewes were isolated from rams for more than one month they reacted to the introduction of a ram with a rapid increase in their plasma LH concentration and a preovulatory LH surge. A relationship was observed between nitric oxide secretion and steroid hormone secretion and LH. This states that the changes in nitric oxide concentrations are necessary for cyclic activity in sheep.

### Camel

**Semen cryopreservation:** Cryopreservation of semen samples artificially collected from adult male camels of Jaisalmeri breed (Dromedary) revealed that pre-freeze sperm motility varied from 47 to 70% in different males. Post-thaw sperm motility declined to 23.5 from 47.5% in individual semen samples with an overall estimated loss of 62.5% of the progressively motile spermatozoa due to freeze thaw process. Based on the criteria to approve semen of 30% or greater post thaw motility, only 37% of semen samples processed in present study qualified for use for AI. Post-thaw sperm motility of same semen sample cryopreserved in duplicate vials in same batch differed significantly may be due to lack of precise control over transition phase during cooling at subzero temperature. At 37°C, the reduction in motility was about 50% than that at 0 h. At 2, 3, 4 and 24 hr after incubation, almost 93, 99 and 100% spermatozoa lost motility. At 4°C incubation, the per cent decline was 17, 30, 35.8, 44.1 and 65.5%, at 1, 2, 3, 4 and 24 hr of incubation.

### Yak

**Reproductive efficiency:** Heatsynch could be successfully utilized for induction of estrus in anestrus yaks to cut short the long postpartum interval. Ovulation following heatsynch protocol was found well synchronized in yaks, and the application of fixed

time AI in this species could be successful. Plasma progesterone profile from individual yaks subjected to superovulatory treatment suggested that a poor superovulatory response in terms of embryo recovery in certain animals was caused by the lysis of corpora lutea before flushing which was carried out 7 days after superovulatory estrus. It was suggested that flushing 5-day post superovulatory estrus could improve the superovulatory response in this species.

**Biochemical studies:** Vitamin A concentration in various vital organs of yak was estimated. Vitamin A in the liver of yak was 45, 016, 910 IU/100 g (41,107 to 47,846) which is almost similar to that of ox liver but lower than that of cattle raised on pasture. In spleen, heart and lung, vitamin A was non-detectable while in kidney, vitamin A was found in traces.

### Mithun

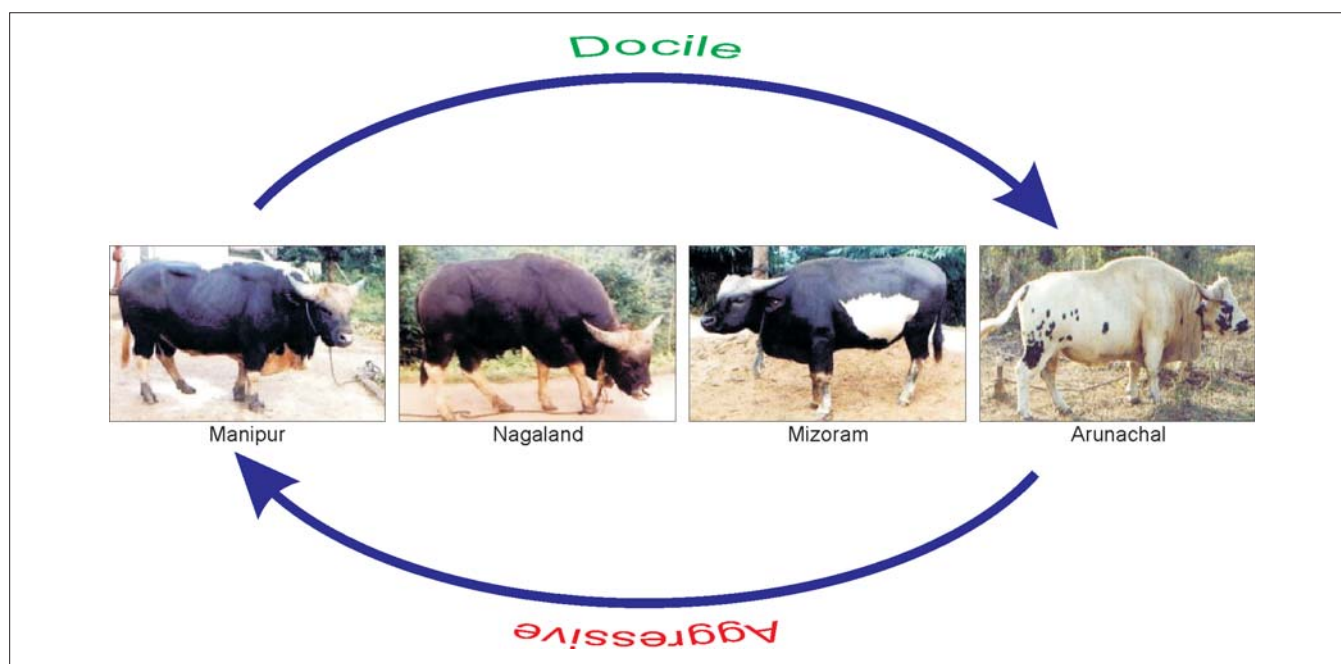
**Growth hormone and temperamental behavior in mithun:** The aim of this study was to verify whether or not plasma growth hormone (GH) concentrations were correlated with temperament (aggressiveness or docility) traits in mithun. Female mithuns from Arunachal, Nagaland, Mizoram and Manipur strains of Medziphema Mithun Farm of the institute, were studied. The temperament score was divided in six-scale points, scale six being very aggressive and scale one being docile. Results indicated that the strain had significant effect on blood GH levels and

### SUCCESS STORY

#### First yak calf born through embryo transfer technology

Yak cow, superovulated with follitrop-V, was used for collection of embryos. Three embryos were successfully collected non-surgically from the donor yak cow. One of the transferable embryos (morula) was transferred to a recipient yak cow whose heat was synchronized with that of the donor. One female yak calf, named Mismo was born to recipient yak cow. This was the pioneering work on yak ETT indicating prospects of implementing ETT in ex-situ conservation as well as for augmenting reproductive efficiency in yak.





temperamental traits.

The temperament scores were tended to decrease with increasing age for all four strains. The highly positive correlation between blood GH concentrations and temperament for all animals regardless of age and strain differences clearly indicated the relationship between blood GH and temperamental behavior in mithuns. Results suggested that peripheral blood GH levels can influence the temperament in mithuns. This is the first report ever indicating the role of endogenous GH on temperamental behavior in livestock species in general and mithun in particular. Selection of animals of commercial importance that is better suited for human handling and management are in more demand than those are difficult to handle and manage. The established relationship between blood GH and temperament in mithuns suggest that blood GH may be used to differentiate mithuns of more docile nature within or among four different strains from the aggressive ones for commercial mithun rearing.

**Preservation of semen:** The colour, consistency and mass activity of fresh semen samples were creamy white, medium and 3 to 4 respectively. The average volume, pH and sperm concentration ( $10^6/\text{ml}$ ) of mithun semen were  $0.6 \pm 0.01 \text{ ml}$ ,  $6.9 \pm 0.03$  and  $425 \pm 48$  respectively. The progressive motility and live sperm count decreased significantly after 36 hr of storage. Whereas, the total sperm abnormality increased significantly over the time of storage and the highest value was recorded at 72 h. The tris-egg yolk diluents could be used to preserve mithun semen at refrigeration temperature. An acceptable level of progressive motility, live sperm count and morphologically abnormal spermatozoa were observed

till 36 h of *in vitro* preservation at  $4^\circ\text{C}$ . However, further efforts are needed to design suitable diluents to extend the preservation time of mithun spermatozoa.

**Cryopreservation:** Ejaculates of good quality (mass activity score 3 or more) were preserved in liquid nitrogen and the progressive motility, live sperm count and acrosomal integrity of mithun sperm were recorded after cryopreservation. In cryopreserved semen approximately 40% progressively motile spermatozoa, more than 50% live spermatozoa and nearly 65% spermatozoa with intact acrosome were observed. In contrast, the morphological abnormalities of mithun sperm increased after cryopreservation and approximately 30% morphological abnormalities were recorded in cryopreserved semen. Following AI, pregnancy was established in 67% mithuns.

### Poultry

**Melatonin studies:** Pinealectomy (Px) and sham operation were done in day-old healthy chicks. The study was run from day-old to day 20 of age. MT was administered @ 5 ppm. Results indicated that Px does not affect gut MT level significantly but a numerical decrease was observed. Exogenous MT administration significantly increased gut MT level at day 20. Exogenous MT administration significantly increased some digestive enzyme levels while Px was accompanied by only a small numerical decrease in digestive enzyme activity. Feed conversion efficiency was significantly better in MT supplemented group compared to non-supplemented group. Pinealectomy resulted in relatively poor FCR compared to control group. MT supplementation also decreased

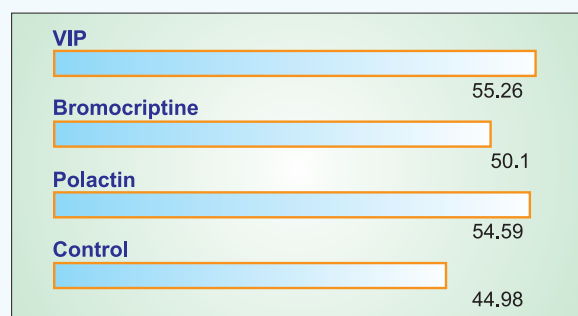




### Prolactin modulation

Investigations at the NIANP, revealed higher than normal levels of circulating prolactin (PRL) in birds during pause days between clutches of egg laying. Hypothesizing that modulating PRL within normal physiological levels would reduce the pause days and increase egg production the following studies were conducted. The PRL levels were maintained within normal physiological range by using 3 approaches (i) administration of bromocriptine, an antiprolactin agent, through feed; (ii) active immunization against PRL; and (iii) active immunization against VIP, the PRL hormone releasing agent.

Overall increase in egg production over the control was highest at 10.28% in birds immunized against VIP as compared to birds immunized against PRL (9.60%) and birds fed with bromocriptine (5.12%). The technique was economically viable. The implications of these results would be of higher significance in country birds that show more intense broodiness/pause days; thus there is a possibility of much greater increase in egg production through modulation of PRL.



Mean percentage of egg production between control and treated Girirani birds from 19-week to 72-week of age

feed consumption/chick/day while pinealectomy increased feed consumption/chick/day. Weight gain in MT supplemented group was also better compared to corresponding control, while Px did not affect the weight gain. Px had no effect on either on jejunal MT level or various digestive enzymes activities, whereas MT administration exerted significant effect on gut MT level, some digestive enzymes and performance of broilers. These results indicated that gut MT level may be manipulated to improve broiler performance.

**Behaviour of apoptotic genes during forced molting:** The role of apoptotic genes, nitric oxide and cytokines in remodeling reproductive tissue during forced molting, was studied in healthy single comb White Leghorn hens. Molting was induced by feed withdrawal (FW) method in the first group; by high zinc (20,000 ppm) feeding (ZnF) in second group along with the remaining was kept as a control. The ovary and oviduct weight reduced significantly during the force molted (FW and ZnF) birds. In first

(FW) group, the reduction was drastic from the 4 DOM and further reaching about 21% of original weight in ovary and 35% of original weight in oviduct on 10 DOM. In the second group (ZnF), a significant reduction in ovary weight was noticed on 5 DOM, which reduced further reaching about 31% of the original weight on 10 DOM. The weight of oviduct also reduced significantly on 5 DOM, which reduced further on 10 DOM attaining about 41% of its original weight. In control birds, there was no significant reduction in ovary and oviduct weights.

Percentage of atresia is comparatively more in ZnF group than the other two groups. The vacuolation was very prominent in the glandular tissue of magnum in first (FW) group, whereas, Zn feeding did not cause much vacuolation on 10 DOM. Constitutive expression of caspase-1 and -2 was observed in ovary and oviduct of control birds. In ovary and oviduct of FW group there was an initial peak in caspase-1 expression that was subsequently down regulated. In ZnF group, the caspase-1 expression showed two peaks during the treatment period in both ovary and oviduct. Caspase-2 expression was upregulated significantly in the ovary and oviduct of ZnF group with two significant peaks during the treatment period. However, there was slight upregulation in the ovary of FW group. Ovarian iNOS expression exhibited different pattern in two methods of molting (feed withdrawal and zinc feeding). In fasting birds, the expression was up regulated significantly at the later stages of molting, whereas zinc feeding increased the iNOS expression from the initial days of molting. The tissue levels of NO also exhibited the similar pattern of iNOS in both methods of molting. In oviduct, the expression of iNOS was higher until end of experimental period in feed withdrawal birds. However, in zinc feed birds the iNOS expression was down regulated at the end. The same pattern was observed with tissue NO levels also in oviduct. The expression of IL 6, IL 8, MIP 1 $\beta$ , and IFN  $\gamma$  mRNAs in ovary and IL 1 $\beta$ , IL 6, IL 8, MIP 1 $\beta$ , IFN  $\gamma$  and TGF  $\beta$ 2 mRNAs in oviduct were up regulated significantly during induced molting by feed withdrawal and zinc feeding, suggesting their role in tissue regression. The constitutive expression of IL 2 and MIP 1 $\beta$  in both ovary and oviduct were negative up to the 40 cycles of PCR.

**Induction of early maturity:** *Hormonal preparations*—Healthy Kadaknath females of the same hatch were administered with progesterone plus estrogen analogues. The treated birds showed nearly three eggs per bird extra as compared to their counter part in control group. Peak egg production was observed in all treated birds after 24–25 weeks onwards. The average egg weight of all the groups followed the similar pattern.

*Non-hormonal preparations:* Healthy *desi* fowl (Kadaknath) from the same hatch were given non-hormonal preparation along with feed from 0 day onwards. First egg production was recorded in 19th week of age only in treated birds. An enhanced pattern of egg



production was found in all the experimental groups, however, maximum egg production (37.3 eggs/bird) was found in birds fed low dose of non-herbal preparation. By adopting this methodology, egg production can be enhanced around 15 eggs per bird.

## LIVESTOCK PRODUCTS TECHNOLOGY

- Methods for preparation of several milk products were standardized
- Test developed for detecting detergent in milk
- Herbal ghee was prepared; it has sensory response similar to market ghee
- Mozzarella cheese was developed from Jamunapari goat milk

### Milk and milk products

Developed *acido-bifidus* **probiotic dahi** and its health benefits for attenuation of colon cancer and dietary hypercholesterolemia and stimulation of body immune system were validated. *Lactobacillus casei* was used in combination with *L.acidophilus* and mesophilic dahi culture 167 (BD4) to prepare **low calorie probiotic lassi**.

A platform test was developed for **detection of detergent in milk**. The developed method can detect detergent up to 12.5 mg/100 ml. A multipurpose device and process was developed for **protein** concentration by dialysis and buffer exchange. **Starch estimation** by enzymatic and polarimetric methods was validated for their applicability in milk. A rapid colorimetric test was developed for detection of **vegetable oil adulteration** in ghee. A number of iron salts were evaluated for their suitability in preparation of **iron-fortified milk**. Buffalo milk exhibited about 10% more **anti-oxidant activity** as compared to bovine milk. Analysis of milk and milk powder samples collected from Southern Region indicated that **organochlorine pesticide residues** were within MRL in milk. Samples from the organized sector scored better for flavour and texture than the samples from the unorganized sector. An improved process was developed for **kheer** mix and rabri. A process for **a lassi-like beverage** was standardized using rennet whey. **Mozzarella type cheese** was developed using skim milk and vegetable oils/fat replacers. A technology was developed for **whey-based sports beverage**. Attempts were made to enhance the shelf life of the *Palada Payasam* to a commercially viable level by retort processing. The retort processed payasam was found to have a shelf life of more than 28 days at 37°C. A process was standardized for retort processed **gasa gase payasam**. The retort processed payasam had a shelf life of more than 6 weeks at 37°C. A prototype unit of continuous **paneer manufacturing** was upgraded. An automatic

### Platform test for detection of detergent in milk developed

A long standing need for developing platform test for detection of adulteration of milk with so called synthetic milk has been fulfilled by focused efforts of scientists. Detergent is known to be one of the critical chemical substances used in preparation of so called Synthetic milk. A colour based test that provides the result within 5–10 min was developed for this purpose. The test can detect presence of detergent in milk up to 12.5 mg/100 ml milk.

Detection of adulteration of 'synthetic milk' in milk. Relatively more blue colour in lower layer indicate presence of 'synthetic milk' (right tube). Relatively more blue colour in upper layer indicate absence of 'synthetic milk' (left tube).



pH sensing and acid dosing control was installed for optimum curdling conditions. A **chhana ball** making machine was integrated with sugar syrup cooking equipment to produce rosogolla in synchronized manner. Conical process vat and thin film scraped surface heat exchanger were suitable for manufacture of **Basundi** for small and large scale manufacture of Basundi, respectively. Basundi could be stored for 5–7 days with acceptable quality at 35°C and for 22–25 days at 7°C on 5 psi/10 min heat treatment. Effluent treatment parameters using combined anaerobic and aerobic treatment techniques were evaluated and standardized. UASB activated sludge bed combination reactor (comb reactor) provided operational economy and efficiency for treatment of **dairy plant effluents**. Three-dimensional structure of buffalo **chymosin** determined for the first time from the crystal. A technology was developed for the preparation of customized **shrikhand** as per consumer's preference based on the starter selection and method of chakka preparation. *Bacillus coagulans* B37 was found to be a potential **probiotic culture** based on *in vitro* functional attributes and *in vivo* trials in mice and could be



### Herbal ghee developed

Ghee is a fat rich dairy product, which is an integral part of our culture. It is mainly used as food and flavouring ingredient. But ghee contains cholesterol, which is one of the suspected culprits in arteriosclerosis

Dairy technologists of ICAR have now developed a process for preparing **herbal ghee** with the addition of phytosterols. The level of herb and other ingredients were optimized using central composite rotatable design (CCRD) of response surface methodology using design expert software. Based on sensory responses generated for different levels of ingredients, the final product formulation was developed. The developed ghee was found sensorily similar to the market ghee. It had overall acceptability score of 85.1 compared to the control (90.84). It complied to all the conditions laid down by PFA and AgMark (BR reading 42, moisture 0.13%, FFA 0.362%, RM value 28.2). The ghee was highly stable (8 days at 80°C) as compared to control ghee (2 days at 80°C) as it contains antioxidants like polyphenols, and terpenoids in addition to phytosterol.



used as a functional ingredient in probiotic foods. Infant stool appeared to be a potential source for isolation of probiotic microorganisms. The bifidobacterial isolates along with existing standard lactic ferments could be exploited for development of **probiotic dairy foods**. Xylanase and cellulase were purified from *B. licheniformis* and its physico-chemical properties elucidated. *Artificial Neural Network* was found a useful tool for predicting sensory based quality of long life milk and milk products and has better prediction ability than kinetic based mathematical models.

**Technology for extended shelf life of mango lassi developed:** Of late there has been a merging of dairy products and fruit beverage markets with introduction of 'juiceuticals' that include hybrid products like fruit based cultured milk beverages. In India *lassi* made out of *dabi* is a widely consumed fermented milk beverage. However, problems like short shelf life, post acidification, whey syneresis etc. hinder the market saleability of *lassi*. Inclusion of mango pulp in *lassi* not only helps in its value

addition but also aids in reducing the post harvest losses in mango.

Ingredients for the formulation of mango *lassi*, were optimized. The shelf life of mango *lassi* was extended using biopreservatives. The individual and interactive effects of milk fat (0.5–6%), sugar (12–17%) and mango pulp (4–14%) on sensory and physicochemical properties of Mango *lassi* were studied. The optimum formulation conditions of milk fat, sugar and mango pulp per kg curd were recommended for the blend formulation. A good quality, highly stable mango *lassi* with less than 1% whey separation was thus obtained using a blend of biostabilisers in combination with small amount of pectin. The shelf life of mango *lassi* was further extended to 50 days at refrigeration temperature using bacteriocin obtained from propionibacteria. The technology developed for manufacture of mango *lassi* with extended shelf life has considerable potential to facilitate commercialization.



**Mozzarella cheese:** Mozzarella cheese was made from Jamunapari goat milk using starter culture method. Pure goat milk Mozzarella cheese had a yield of  $13.37 \pm 0.21\%$ . Sensory score for appearance, body, texture and flavour was better in respect of vacuum packaged cheese compared to aerobic packaged cheese. The shelf life of the cheese was 14 days at refrigeration temperature and it can safely be stored in polyethylene bags under vacuum packaging. However, the product acceptability was very low on day 14th for the cheese stored under aerobic packaging system.

### Meat and Meat Products Technology

**Value added meat products: Chicken vada** was developed by using chicken emulsion or chicken byproducts incorporation. Linseed (*til*) and soya (whole seed) could be successfully incorporated to produce nutritionally superior **emulsion based meat products**. A beneficial incorporation of capsicum, carrot

- Process developed for use of chicken neck in value added meat products
- Low acid goat meat pickle prepared
- Technology developed for preparation of different products from mithun meat
- Black pepper extract proved effective preservative for chicken gizzard snacks
- Rabbit hair (10%) blending with Bharat Merino sheep wool made woollen products more soft
- Different products were prepared from mithun leather





#### Utilization of fluid goat milk whey in goat meat products

Fluid goat milk whey in place of ice was used in goat meat nugget formulation. Evaluation of physico-chemical, textural and sensory quality properties of the nuggets revealed increase in emulsion stability rate, ash content and pH value. The 100% whey replacement did not produce any adverse effect in nugget's sensory properties and nuggets were highly acceptable to the panelists, suggesting that valuable animal product, often wasted can be used to create a highly acceptable and valuable food product at minimal cost.

and radish in mutton nuggets and patties was achieved.

**Low acid goat meat pickle:** Low acid goat meat pickle was prepared using deboned meat from Sirohi goat of around 1.5 years age. After seven days of maturation period, the product was evaluated at an interval of 15 days up to 2 months of storage at room temperature ( $32 \pm 0.5^\circ\text{C}$ ). The pH and titrable acidity of the low acid pickles was  $4.92 \pm 0.02$  and  $0.68 \pm 0.04$  whereas in standard goat meat pickle, these values were  $4.73 \pm 0.02$  and  $0.74 \pm 0.03$ , respectively. Microbiological counts and sensory quality traits remained satisfactory throughout the storage period. Low acid pickle had significantly lower sourness and higher overall acceptability compared to the conventional meat pickle.



#### Mithun

Technology for preparation of nuggets, patties, meat blocks and dried meat powder from mithun meat were standardized in the laboratory. All meat products were equally acceptable to the mithun meat consumers. Organoleptic tests showed high rate of acceptability score of 5 to 6 in the scale of 1–7.



#### Chicken

**Meat products:** A process was developed for use of **chicken necks** in value added products. **Chicken soup** and chicken pickle were developed from deboned frames. **Chicken meat spread**, a highly acceptable variety product was successfully developed. Several suitable alternate formulations for low sodium and high **fibre meat products** were developed.

#### Value-added product from low-value spent hen meat:

Efficient and economical disposal of spent (culled) hens is a major problem to layer farmers because of less demand of its meat characterized by toughness and excessive carcass fatness. Thus, a process for the development of premium value-added chicken meat block from low-value spent hen meat and edible by-products was optimized and its shelf-life assessed. Less preferred spent hen meat (60%) and SGH (15%) in combination with non-meat ingredients and permitted food additives (0.5% sodium tripolyphosphate and 0.02%  $\alpha$ -tocopherol acetate) could be processed into a premium comminuted meat block. Incorporation of 0.4% lactic acid in the formulation appeared beneficial in extending the refrigerated ( $5 \pm 1^\circ\text{C}$ ) shelf-life of the product up to 15 days without imparting perceptible sourness, comparable to that of vacuum-packed samples without acidulation, as against 10 days for control group. Both control and vacuum-packed samples had a shelf-life of 90 days under frozen ( $-18^\circ\text{C}$ ) storage.

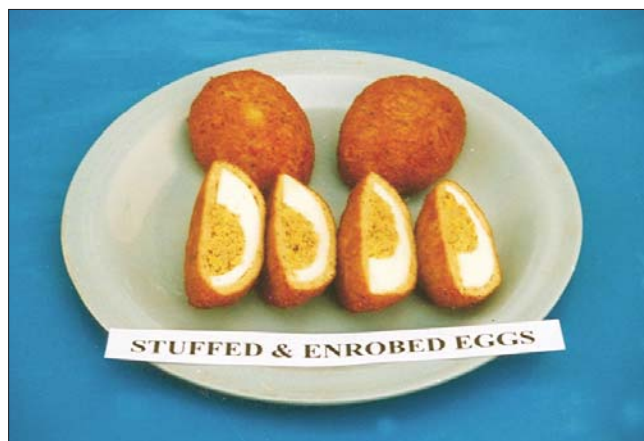
**Phyto-extract as a preservative for chicken gizzard snacks:** Processing of chicken gizzard snacks with 0.4% level of BPE (black pepper extract) rendered good quality product till 21 days of refrigerated ( $4 \pm 1^\circ\text{C}$ ) and 42 days of frozen ( $-18^\circ\text{C}$ ) storage. Post-processing infrared treatment for 60 sec improved microbial quality of gizzard snacks.

**Assessment of chemical and mycotoxin residues in spent hen tissues:** Determination of the residual level of some heavy metals revealed that lead content in market samples of chicken liver or egg was higher (0.25 ppm) than that of spent hen lean meat (0.2 ppm). Arsenic in muscle, liver and adipose tissue ranged from 0.20 to 0.25 ppm. The residue of BHC in muscle ranged from 0.05–0.2 ppm whereas in liver and adipose fat it was in the range of 0.02–0.3 ppm and 0.02–0.05 ppm, respectively. The samples of tissues collected from commercial outlets had relatively higher level of residues than that of institute farm samples. The level of DDT was recorded to be 0.02–0.15 ppm in muscle, 0.05–0.3 ppm in liver and 0.1–0.35 ppm in adipose tissues. The residual level of malathion was 0.02–0.05 ppm in liver only and was not detected in muscle and adipose tissue. Aflatoxin B<sub>1</sub> was not detected in any tissue analysed. However, the residues of heavy metals and pesticides analyzed in this study in both institute farms and market samples were below MRL.



### Egg products

**Value-added egg product:** Eggs stuffed with 1 : 1 or 1.5 : 1 yolk and meat mixture, respectively, and coated with a thin layer of chicken meat emulsion were liked most and had a refrigerated ( $4 \pm 1^\circ\text{C}$ ) shelf-life of 18 days in vacuum and 16 days in aerobic pack with satisfactory microbiological and sensory quality.



### Surveillance of bacteriological quality of chicken eggs:

Evaluation of the bacteriological quality of chicken eggs collected from selected poultry farms of Uttarakhand and Haryana and in marketing (wholesale/retail) channels revealed that farm fresh eggs had lower aerobic ( $\log 3.1\text{--}3.7/\text{cm}^2$ ) and coliform counts than those collected from wholesale/retail outlets. The incidence of *Salmonella* in chicken eggs was 1.9% while that of *E.coli* 9.7%, and their occurrence in feed sample was 2% and 18.5% respectively. *Salmonella* detection using cell lysate PCR appeared as a quick and reliable method.

### Wool, fibre and hide

The evaluation of farm bred Chokla sheep wool samples revealed that wool has become very fine (26.5 micron) as compared to the Chokla sheep wool from the field area (30 micron). Avikalin

### Mithun leather products

Mithun hides were processed for the first time and different types of leather products were produced. These were of very high quality. Leather processed with intact hair was a stuff of excellence having good usability as outer cover of sofa as well as carpet. The mithun leather could be used for bag leather, shoe upper, garment leather etc. with superior finish.



sheep wool is also more or less similar to it. Both the wools have staple length of around 40 mm, which is less from processing point of view.

**Angora hair:** Rabbit hair (10%) was blended with Bharat Merino sheep wool to make the woollen products more soft. About 5 Nm yarn was spun on woollen systems for its conversion in to shawls. The final product after wet processing was more soft and white than woollen product. Woollen yarn (4 Nm) obtained from Bharat Merino sheep wool was dyed into yellow, black, red and green colours is being used for the preparation of standard quality blankets. Finished blankets have shown excellent appeal, softness and warmth.