

# Livestock and Poultry Improvement and Management

# Livestock information management

A generalized and flexible data processing system was developed for management and analysis of field survey data on characterization of animal genetic resources. It works for all the livestock and poultry species and accepts any type of questionnaire format. Analysis of the data can be performed on the basis of districts, animal classes and the strata defined on the management practices. Herd data can be analysed for a species, a district and a village. The user can view and extract the raw data for further analysis using available commercial software.

# Phenotypic characterization and evaluation of indigenous breeds

**Kenkatha:** This cattle breed is distributed in Lalitpur, Hamirpur, Chitrkoot and Banda districts of Uttar Pradesh and Tikamgarh district of Madhya Pradesh. Animals of this breed are mainly used for draught purpose and milk, and are of small size having grey and white body. Head is short and broad, horns curved, face short and disc shaped, hump and dewlap moderate, ears horizontal, muzzle, eyelids and hump black, tail moderate in length, and udder and teats small but squarely placed. The average measurements (cm) for body length, height at wither and heart girth were 101.60, 103.33 and 136.86 respectively. The birth weight, daily milk yield and calving interval ranged from 12 to 15 kg, 0.5 to 2.0 kg and 15 to 20 months respectively. The load carrying capacity of a pair of bullock was 5 to 8 q with 4 to 6 km/ hr speed.

**Hallikar:** Hallikar animals are distributed in Mysore, Mandya, Bangalore, Kolar, Tumkur, Hassan and Chaitradurga districts of Karnataka. Milk yield of Hallikar cows is low but their bullocks are famous for draught capacity. The chest girth, body length and height at withers (cm) were 161.02, 134.44, 132.15, respectively, in adult males, and 148.14, 126.67 and 124.51, respectively, in adult females. The coat colour of animals is grey to dark grey. The head is prominent with typical horn pattern i.e. emerging from top of poll, goes back up to half of its length and thereafter bends slightly forward and slightly inward at tips. The average milk yield of cows varies from 0.5 to 3.0 kg/day. The load carrying capacity of a pair of bullock ranged from 0.5 to 5.0 tonnes for 5

- Flexible data processing system provides even raw data for further analysis
- Under phenotypic characterization programme several indigenous breeds of cattle, buffaloes, sheep, goat, poultry, camel and horses were studied in their home tract
- Twinning in Kutchi goats increased up to 50% by supplementary feeding
- Breed specific marker was identified for Surti buffaloes
- Twinning in Malpura, Marwari and Bharat Merino was not found linked with FecB gene
- Marwari equine population has high genetic variability that equine breeders may exploit
- Genetic bottleneck was not observed in Ankleshwar and Punjab Brown poultry in past populations
- Juvenile body weight of naked neck was superior to normal birds
- HSRBC and HCMI lines showed higher Newcastle disease vaccine response
- Under *ex-situ* conservation programme frozen semen samples of cattle, buffaloes, goats, sheep and camel were preserved in genebank
- Molecular genetic characterization of wildlife species was initiated

to 15 km in a day.

**Banni:** These buffaloes are prevalent in Nakhatrana, Khavda, Haji Peer, Bhrindiara, Hodka, Zarpara villages. Banni area has 35,000 to 40,000 buffaloes. They are usually black and few (about 5%) are brown, which are good milk yielder. Built medium to large, morphologically these buffaloes have distinct coiling of the horn, which is aligned vertically to the body and coils inwardly. The animals are wedge-shaped with heavy backs and are maintained under (almost) zero input system. Their age at first calving is 3 to 4 years; dry period, 1 to 1.5 months; lactation length, 10 to 10.5 months; calving interval, 14 months; average daily milk yield, 10 kg, and fat in milk, 7-8%.

**Sonadi:** These sheep are medium to large in size. Average body weight, body length, height at wither and chest girth of rams and ewes were 42.2 and 29.7 kg, 72.6 and 64.3 cm, 74.1 and 66.3 cm, and 81.7 and 73.9 cm respectively. The face is white or light brown, and this colour may extend up to neck. Ears are small to medium in length. Both the sexes are polled. The tail is thin and



of medium length. Body is covered with white, open and coarse fleece.

**Nali:** The Nali sheep is found in Ganganagar, Churu and Jhunjhunu districts of Rajasthan, and southern parts of Hisar



Sonadi sheep



Sirohi goats are reared for milk and meat purpose



Kutchi goats

district of Haryana. The animals are medium in size. The average body weight, length, height at wither, chest girth were 38.85 kg, 68.95cm, 69.16 cm and 80.03 cm, respectively, in males, and 31.38 kg, 64.17 cm, 64.39cm and 75.95 cm in females. The face is light brown and the skin is pink. Both sexes are polled, ears are medium and tubular, tail is short to medium and thin, fleece is white, coarse and dense, and wool is of reasonably good carpet quality. Age at first breeding in males ranged between 12–18 months with a breeding life of 5–6 years. In ewes, age at first lambing ranged between 18–24 months. The lambing rate was about 80% and lambing interval 12–18 months. An ewe produced 4–8 lambs in its lifetime. The Nali sheep are maintained for mutton and wool production. Average greasy wool production was about 1.5–2.0 kg per annum in 2–3 clips.

*Sirohi:* Sirohi goats are found in the Sirohi, Ajmer, Chitorgarh, Rajsmand and Udaipur districts of Rajasthan and the adjacent districts of Gujarat state. The animals are reared for milk and meat purpose. Three types of colour variants, viz. brown spots, brown spots but with white patch on the face and uniform light brown, were observed. The estimated average means of body height, body length, chest girth (cm) and body weights (kg) were 77.62, 77.14, 77.62 and 43.05, respectively, in adult males, and 75.52, 75.81, 76.37 and 38.35 in adult females. Polled animals are present in Devgarh area of Rajasmand district. The flock size varies from 10 to 100. Animals are kept on pasture grazing; stall fed animals are rarely seen. Females show maturity at about 12 to 18 months of age. The breeding seasons are March–April and September–October. Kidding is once in a year. Twining is only in well fed goats (10–20%). Milk yield varies from 0.5 to 2.0 kg.

**Kutchi:** These goats are found in the villages of Banaskantha, Patan and Kutch districts of Gujarat state. Kutchi goats are of medium size and are uniformly black with shinny light grey skin. Nose is slightly Roman. Muzzle, eyelids and hoofs are black. Eye pupil is brown. Horns are small (2 cm) to long (30 cm), corkscrew type, greyish black and curved upward. Ears are pendulous, small (4 cm) to long (24 cm) and black. Ears with white spots or white base are also seen. Forehead is convex with curly tuft hair particularly in male. The average body weight of male and female goats were 44.57 and 35.58 kg, respectively. They mature at 12 months of age. Twinning is 20%, however, with supplementary feeding it increases up to 50%.

**Marwari:** Marwari horses constitute an elite group of indigenous horses, which are known for their sturdiness, swiftness, elegance and beauty. The phenptypic characters of Marwari breed were established. True-to-breed Marwari horses (114) comprising 98 mares and 16 stallions from 7 different locations were evaluated.

**Ankamali:** This pig derives its name from the Ankamali block in the Ernakulum district of Kerala. These pigs are also





Ankamali pigs are suitable for backyard pig farming

found in Karnataka, Tamil Nadu, parts of Maharashtra and Andhra Pradesh. They are raised in backyard on kitchen waste and other agricultural and industrial byproducts. They are black often with white patches. They possess a long face, tapering towards the nostrils and bulging can be clearly seen at the joints of the jaw. Their body is medium sized and compact with pot belly as compared to exotic pigs. The hair on the neck and part of the back are thick, long and bristle but those on the sides and the flank are thinner and shorter. Head and shoulder are heavier as compared to hindquarters, back is slightly arched and rump is dropping. Tail is thin and long. Females possess 6-12 teats. Males are generally heavier than the females. The average litter size at birth is 8.0 and litter size at weaning is 5.0. The average age of slaughter is 9 months. Normally pigs over 40 kg are sold for slaughter. The dressing percentage is 68.

# Molecular genetic characterization of indigenous breeds

*Cattle and buffalo:* At the IVRI Izatnagar, molecular characterization of cattle and buffalo breeds was undertaken.

- Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) studies in cattle breeds (Jersey, Sahiwal, Hariana and Tharparkar), crossbred cattle (Holstein Friesian × Hariana) and buffalo breeds (Murrah, Bhadawari and Mehsana) with Hae III and Rsa I, revealed that the MHC class I gene is highly polymorphic in studied cattle and buffalo populations.
- Alleles (17) selected on the basis of PCR-RFLP and single stranded conformational polymorphism (SSCP) were cloned and sequenced for the characterization of MHC class I gene in 3 buffalo breeds (Murrah, Mehsana and Bhadawari) and indigenous (Sahiwal) and crossbred (Holstein Friesian  $\times$ Hariana) cattle.
- Crossbred cattle calves with certain genotypes showed better cell-mediated immune responses suggesting thereby that the

# Tho-Tho cattle

This cattle breed is distributed in nearly all the districts of Nagaland state. The breed is also known as Ameshi, Sheapi, Chokru, Tseso etc. They are reared primarily for meat and dung production and occasionally for milk by all the major tribes of Nagaland. The animals have social importance to Naga tribes particularly for dowry and other tribal rituals. Freerange system without any supplementary feeding is followed. However, farmers offer common salt to their animals once in a week. During non-cultivating season (December to March), animals are left free for grazing and do not return to their homestead. During cultivating season (April to November), animals are grazed during day time and are brought back for night. Natural breeding is practised. The animals are very hardy and believed to be disease resistant. However, sporadic cases of FMD, tick infestation and gastro-intestinal nematodiasis are reported. In adult animals body length, height and heart girth ranged from 99 to 109 cm, 114 to 117 cm, and 139 to 147 cm, respectively. The milk production is 0.5 to 1.0 litre/ day, lactation length 3 to 4 months, age at first calving 3 to 4 years, service period 60 days, calving interval around 1 year, life span 15–16 years, and lifetime production is 8–10 calves.



calves of these genotypes might respond well to the natural infection with Brucella organisms.

- A fragment of insulin like growth factor binding protein 3 (IGFBP3) gene was of 654 bp size in Surti buffaloes, as compared to 655 bp in Murrah and Bhadawari breeds, which may be used as breed specific marker of Surti breed.
- A 280 bp amplified product of insulin like growth factor 1 (IGF1) gene was found to be polymorphic in goats and monomorphic in buffaloes by PCR-RFLP, SSCP and sequencing techniques.
- The cDNAs of integrin beta 2 (ITGB2) gene of buffalo were cloned and sequenced.
- RAPD-PCR analysis of buffalo DNA, based on 20 primer sequences revealed that 11 to 35% polymorphism obtained in buffalo



genome was able to segregate the high and low service period animals with 39% dissimilarity by cluster analysis. However, sub-groups of animals with high and low age at first calving could not be segregated by RAPD-PCR analysis. Some SSR sequences were able to show 44% dissimilarity in DNA band patterns of high and low milk producing buffaloes.

**Yak**: The molecular genetics work was initiated, and the random amplified polymorphic DNA (RAPD) assay, using 10 random decamer oligonucleotide primers, was conducted to study the genetic similarities and divergence among five types (Common, Bisonian, Bareback, White forehead and Hairy forehead) of Indian yaks.

**Sonadi**: Microsatellite analysis of Sonadi sheep with 15 FAO (USA) proposed ovine specific microsatellites exhibited wide range of genetic variability depicted by allele number varying from 3 to 9, observed heterozygosity from 0.181 to 0.956, expected heterozygosity from 0.275 to 0.851, and polymorphism information content (PIC) from 0.252 to 0.809. In addition various average genetic variability measures, viz. allele diversity (5.8), observed heterozygosity (0.567), and gene diversity (0.667) showed high genetic variability in Sonadi sheep.

*Marwari:* The microsatellite based molecular genetic profile of Marwari sheep displayed a high level of genetic variation. The observed alleles, effective alleles, observed heterozygosity, expected heterozygosity and polymorphism information content (PIC) values were estimated.

**Kenguri, Bellary, Bandur and Hassan sheep:** Genetic characterization of these sheep breeds by using the microsatellite markers revealed that the effective number of alleles was lesser than the observed number of alleles. The mean observed heterozygosity was significantly lower compared to expected heterozygosity. The average PIC values were  $0.70\pm0.03$ ,  $0.66\pm0.03$ ,  $0.63\pm0.04$  and  $0.66\pm0.04$  in these populations.

**Biotecnological studies on FecB gene:** The *FecB* mutation was analyzed in Garole  $\times$  Malpura (GM) cross (144), out of which 14 were homozygous (*Fec*B<sup>BB</sup>), 89 heterozygous (*Fec*B<sup>B+</sup>) and 41 non-carriers (*Fec*B<sup>++</sup>). Malpura, Marwari and Bharat Merino were found non-carriers for *Fec*B mutation. Twinning in Malpura (42), Marwari (20) and Bharat Merino (20) was not found linked with *Fec*B gene.

**Barbari:** The number of alleles observed varied from 2 to 10 with an overall mean of  $5.542 \pm 2.167$ . The average expected gene diversity within the population ranged from 0.171 to 0.814 with an overall mean of  $0.584\pm0.191$ . Eleven loci showed significant heterozygote deficiency in the Barbari goat population.

**Jamunapari:** Genetic analysis at 23 microsatellite loci revealed substantial genetic variation. Average polymorphism and expected gene diversity in the population were  $1.066 \pm 0.510$  and  $0.528 \pm 0.237$ , respectively. Population showed fairly high level of inbreeding

#### Mewari camel

The Mewari breed is one of the important indigenous breeds of camel, commonly found in South Rajasthan and adjoining Malwa area of Madhya Pradesh. This breed is not being maintained at any farm of the central/state government. A survey was, therefore, conducted and the breed was characterized in the breeding tract itself. The major breeding tract of the breed encompasses Udaipur, Chittorgarh, Rajsamand districts in Rajasthan and adjoining Neemuch and Mandsour districts of Madhya Pradesh. The breed can also be seen in Bhilwara, Banswara, Dungarpur districts and Hadoti region of Rajasthan, which can be considered as a minor breeding tract of the breed. The tract consists of hills of the Aravali in Mewar area. The population of Mewari breed is about 0.025 millions (based on 1997 livestock census, Government of Rajasthan) in the major breeding tract.

Mewari camels are stouter and slightly shorter than Bikaneri. They have strong hindquarters, heavy legs, hard and thick foot pads. They are well adapted to travel and carry loads across hills. The body hair are coarse, which protect them from the bites of wild honeybees and insects. The body colour varies from light brown to dark brown but some animals are almost white, such variation in body colour is generally not seen in other breeds of camel. The head is heavy, set on a thick neck. Unlike the Bikaneri camel, the Mewari camel has no 'stop', but its muzzle is loose. Ears are thick and short, set well apart, tail is long and thick. In females the milk vein is prominent and the udder is well developed.

# $(f = 0.189 \pm 0.049)$ and global heterozygote deficit.

**Attapady:** Genotypic characterization of Attappady goats revealed high level of genetic variability depicted by wide range of Levene's and Nei's expected heterozygosity values, PIC estimates and mean number of alleles. Observed heterozygosity average (0.546), mean expected heterozygosity (Levene's 0.616, Nei's 0.609) implied a higher genetic variability within breed.

**Bhutia:** These horses are bred and reared for riding as well as pack purposes all along the Tibetan border and the sub-Himalayan tract from Punjab to Darjeeling. The genetic characterization using 25 microsatellite markers showed that the mean observed and effective numbers of alleles at all loci were 5.64 and 4.62 respectively. The mean observed and expected heterozygosity at these loci was  $0.56 \pm 0.07$  and  $0.79 \pm 0.05$  respectively.

**Marwari:** Molecular characterisation of these horses was attempted using 26 polymorphic microsatellite markers, known in exotic breeds of horses. Of these, 3 markers were monomorphic in Marwari horses. DNA polymorphism studies revealed high level of heterozygosity and low level of heterozygosity deficit in Marwari horse population, which reflect high genetic variability in Marwari equine population that can be exploited by horse breeders for planning breeding strategies for its conservation. The present



Marwari equine population did not reveal any recent bottleneck, which is very informative and important for equine breeders. To conserve the germplasm of Marwari, technique for cryopreservation of semen of Marwari stallions was standardized.

Bikaneri, Jaisalmeri and Kachcchi camel: DNA samples from Bikaneri, Jaisalmeri and Kachchhi camel breeds were analysed for allelic status at 8 microsatellite licu. The annealing temperature and PCR amplification was optimized for 18 primer pairs. Microsatellite loci, viz. VOLP 32, LCA 37, LCA 23 LCA 77 and CVRL 08, were found monomorphic in indigenous camel breeds. The primer CVRL 07 amplified polymorphic band in 3 Indian breeds. The allele size ranged from 272 to 305 bp. The observed and expected heterozygosities in the Bikaneri, Jaisalmeri and Kachchhi breed were 0.792, 0.4, 0.727 and 0.545, 0.64, 0.731 respectively. The PIC ranged from 0.518 to 0.686. At CVRL 05 locus 4 alleles each were amplified in the size range of 174 to 155 bp in Bikaneri and Jaisalmeri camel and 3 in Kachchhi camels. The observed and expected heterozygosity in Bikaner, Jaisalmeri and Kachchhi breeds was 0.55, 0.57, 0.615 and 0.670, 0.536, 0.522 respectively. The PIC ranged from 0.404 to 0.611.

**Molecular cloning, characterization and promoter analysis of the camel milk protein gene(s):** The a-lactalbumin gene promoter fragment was amplified using camel genomic DNA as template. Polymerase chain reaction gave a clean band of about 880 bp. The PCR product was characterized. Results of Hae III digestion differed from that of the Arabian alpha lactalbumin promoter sequence. The gel pattern of *Hae* III digestion showed 4 bands. The Arabian alpha lactalbumin promoter sequence contains only two restriction site for *Hae* III but our Indian camel population showed 3 restriction sites for the same promoter. Therefore, it can be said that the promoter sequence of Indian camel population has an additional *Hae* III restriction site at about 450 bp. Hence it can be said that the amplified sequence is of a-lactalbumin gene promoter but with polymorphism for *Hae* III.

**Ankleshwar:** The number of alleles observed across the microsatellite loci studied, varied from 4 to 11 with an overall mean of 6.44 in Ankleshwar poultry. PIC indicated the high polymorphism across the loci with an overall mean of 0.623. The average expected gene diversity ranged from 0.304 to 0.843 with an overall mean of 0.670. Genetic bottleneck was absent in the past population of Ankleshwar poultry.

**Punjab Brown**: Microsatellite loci, evenly distributed throughout the poultry genome, were used for generating the data for inferring population genetic parameters. The average number of alleles are 8.05 and average effective number of alleles 4.34. The average expected and observed heterozygosity are 0.74 and 0.61 respectively; and population is in Hardy-Weinberg Proportions in approximately

## Genetic diversity in Indian goats

Indian goats make up 20% of the world's goat population. Indian subcontinent contains 20 well-characterized goat breeds, which vary in their genetic potential for the production of milk, meat and fibre, disease resistance, heat tolerance, and fecundity. The molecular characterization of Indian goats from different geographic regions was done by using mtDNA sequence data from the HVRI region, and diversity and differentiation analysis by microsatellite marker. The first analysis of Indian goat mtDNA diversity in 10 breeds revealed significant genetic structure among them. Two novel additional lineages, D and E, were observed, showing that considerable additional diversity exists within Indian domestic goats. All the examined domestic goat lineages fall in single monophyletic group that was distinct from available wild goat sequences. The lineages contributing to domestic goats were, therefore, derived from unknown population that may now be rare or extinct. Further investigations of wild goats and archaeological specimens are therefore needed to investigate these ancestors. Genotypic data from 17 microsatellites were used to assess genetic diversity and relationship among 8 Indian goat breeds. The highest observed heterozygosities were observed in Jakhrana goats. The phylogenetic tree and the plot for principal component analysis grouped the Indian goats according to their geographic origin and distances between populations were significantly different from each other.



Considerable additional diversity exists within Indian domestic goats

50% of the loci studied. The mode shift test revealed that the population has not experienced any genetic bottleneck in the last few generations.

Genetic selection and breeding for better performance in specialized naked neck male and female broiler population: Two naked neck pure broiler strains having naked neck gene (NNWP and NNCP) have undergone specialized selection programmes over a period of 10 generations. The fertility percentage was 82.2% and hatchability on FES (fertile egg set) was 77.5 in



NNWP. Corresponding values in NNCP were 81.9 and 76.8. The selection criterion in NNWP and NNCP was primarily based on the high 5-week body weight in last 2 generations. The selection criterion in earlier generations was mainly high 6-week body weight. The superiority of juvenile body weights of naked neck over normal broilers at different ages was observed in this generation in both the populations. The combined naked neck birds (homozygous + heterozygous) showed superiority over normal progeny in both lines for dressing percentage and cut-up-parts. The naked neck birds of both the strains, further exhibited superiority for less feathers and abdominal fat at 6 weeks of age.

Genetic characterization and improvement of colour broiler lines: At the PDP, Hyderabad, PB-1 and PB-2 synthetic pureline broiler populations have been maintained for production of a multi-colour commercial broiler for intensive farming. In the S-14 generation of male line, age at sexual maturity, egg weight at 32 and 40 weeks and egg production up to 40 weeks of age were 154.6 days, 54.8 g, 59.8 g and 63.3 eggs, respectively. The corresponding values for the same traits in the S-14 generation of female line were 151 days, 52.5 g, 58.0 g and 65.4 eggs, respectively. The next generation of both the lines were reproduced. Selection differential for 5-week body weight was 165.7 and 109.7 g with an intensity of selection of 1.16 and 1.31  $\sigma$ , respectively, in the male and female lines. The fertility and hatchability on total and fertile eggs set were 72.7, 62.8 and 86.4%, respectively. The primary trait (5-week body weight) in S-15 generation was improved by 26.6 g over previous generation in male line while it remained unchanged in female line. For assessing the genetic gain in these lines, a control line was maintained.

Naked neck (Na) and dwarf gene (dw) lines for use in tropical poultry production: Two distinct populations carrying naked neck and dwarf genes in broiler background were maintained for use as a resource population in tropical broiler breeding programmes. In NG-03 generation of naked neck line, the average fertility and hatchability on total and fertile eggs set were 83, 61 and 74%, respectively. The average 6-week body weight was 917 g with selection differential of 175.2 g and intensity of selection of 1.15  $\sigma$ . The age at maturity, egg weight at 28, 32 and 40-week, and 40-week egg production were 171.8 days, 54.4 g, 57.5 g, 62.3 g and 51.8 eggs, respectively. In DG-03 generation of dwarf line, the corresponding values were 162.3 d, 49 g, 51.3 g, 55.8 g and 57.1 eggs, respectively. The average 6-week body weight was 601 g with a selection differential of 85.2 g, and intensity of selection was 0.78  $\sigma$ .

*Genetic selection for immune response in naked neck population.* In broiler pure lines, direct or indirect selection of resistant strains, general immunocompetance and marker assisted selection, are being considered as promising breeding strategies

#### Genetic characterization of wildlife

Work on molecular genetic characterization of wildlife species was initiated at the IVRI, Izatnagar. Partial mitochondrial 12S rRNA genes of peacock (Pavo cristatus), Indian wolf (Canis lupus lupus), hog deer (Axis porcinus) and Himalayan musk deer (Moschus chrysogaster) have been cloned and sequenced. The wolf 12S rRNA sequence exhibited 100% homology with that of dog. The peacock and poultry (Gallus domesticus) sequences were not similar, indicating potential use of PCR – RFLP to differentiate these species.

against a number of infectious diseases each with different initiations.

Initial results of the study on the antibody response to Newcastle disease (ND) vaccine at 14, 28 and 42 days of post vaccination, showed that among fixed effects, hatch and genotype were significant at 14, 28 and 42 days of age for vaccine response. No significant difference between sex was observed.

# Transgenesis and reconstitution of poultry species

**Using ex-vivo embryo culture system.** The chick has been used as a model system since the beginning of development biology and the ability to make the transgenic modifications/ reconstitution of the endangered poultry species, would be a useful tool for these studies. Understanding basic development of the chick has relevance to both medical and poultry science research. Following hatching observations were recorded in embryo culture system of chicken:

- Orientation of embryo during 20–21 days of incubation majority of embryos were positioned on left (49.48) followed by dorsal surface uppermost (23.71), right side (21.65), ventral surface upper most (3.09), and vertical (2.06).
- (ii) Membrane penetration almost 82.47% embryos penetrated the membrane, 12.37% embryos failed to penetrate the membrane and 5.15% were in dropped condition.
- (iii) Out of total embryos hatched, 65.97% had regular breathing, 22.68% irregular/infrequent breathing, 7.21% had shallow breathing and 4.13% had rapid breathing. No breathing, vocalizing, deep, and gasping were absent during 20 to 21 days of incubation.
- (iv) Chorioallantoic membrane was found becoming detached in 43.29%, followed by well-attached-to-shell (31.95%), poorly attached (20.61%), and slight hemorrhage at shell (4.12%); and high-up-shell and pulse visible conditions were not observed.
- (v) Maximum number of embryos (70.10%) in EC-system showed none visible urates followed by a little (15.46%), some (10.30%) and a lot (4.12%).
- (vi) Embryos exhibited no yolk sac in 41.23%, followed by



34.02% of about 1 cm<sup>2</sup> visible, about 2 cm<sup>2</sup> visible 12.37%, more than 2 cm<sup>2</sup> visible 10.30%, connecting yolk sac 2.06%, and zero percentage to either side of embryos.

- (vii) Climax activity (45.36%), followed by some activity (40.20%), activity of legs (5.15%), head activity (3.09%), and 2.06% each of rapid burst, body and no activity.
- (viii) Right feet or legs were visible in 49.48% followed by visibility of left feet or legs (21.64%), neither feet/legs visible (19.58%), feet/leg between head and shell (8.24%) and both feet/leg visible (1.03%).
- (ix) Dry allantoic fluid was revealed in 49.48% followed by moist (28.86%), wet (10.30%), very wet (4.12%), milky (1.03%), clear (2.06%) and yellow allantoic fluid (4.12%).
- (x) In embryo culture system maximum number of embryos showed horizontal position of beak (45.36%) followed by beak down and not visible (19.58%), beak up (15.45%), feet over beak (7.21%), beak under thigh (6.18%), beak towards shell (4.12%), beak under yolk (1.03%), and body head down (1.03%).
- (xi) Comparative embryonic development at various stages of incubation in *ex-vivo* embryo culture and normally incubated eggs, revealed that only embryo culture system can provide the opportunity to see the development of same embryo continuously each and everyday, during complete incubation period.

In quails semi-quantitative RT-PCR method revealed that myostatin gene expression was at lower levels from E2 to E6 stage of broiler embryo. At E7 stage, highest expression was observed in breast muscles, whereas, liver and brain showed lower expression. Heart did not show myostatin mRNA expression at E7 stage. At day 9, all the organs showed myostatin expression but higher levels of expression were observed in leg and breast muscles and intestine. From E12 to E18 stage the expression of myostatin lowered in all the tissues. One of the siRNA synthesized by *in vitro* transcription was used for transfection in primary CEF. The negative control without siRNAs were also kept. The transfected cells showed marked morphological differences like rounding, aggregation and vacuoles formation from that of control cells from 18 hr onwards.

In G3 generation of immunodivergent lines of layer, HA titre differed between lines whereas the serum lysozyme and IgG levels did not. The genetic diversity between divergent lines was estimated as  $0.04\pm0.02$  by RAPD-PCR technique.

The overall mean for SRBC response ( $\log_2$  titre), CMI (% thickness), serum lysozyme (g/ml) and IgG (mg/ml) levels were studied in immunodivergent broiler lines. The heritability estimates of respective traits were 0.10±0.05, 0.44±0.17, 0.06±0.04 and 0.09±0.05. The candidate genes, viz. BL-BII, IFN- $\gamma$ , IL-2 promoters,

IL-2 R  $\gamma$  chain genes and TGF-B3 were analysed. PCR-RFLP of IFN- $\gamma$  with *Tsp* I enzyme showed genotypes AB and BB. *MnII* PCR-RFLP of IL-2 promoter showed genotypes AA, AB and BB. IFN- $\gamma$  gene promoter (669 bp) was cloned in pGEMT vector. Amino acid sequences of IL-2 exons of SDL were also analysed. Kinetic and differential expression of iNOS, MIP-1B, IL-2 and IFN- $\gamma$  genes by RT-PCR and real time PCR were studied in HCMI and LCMI lines. Vaccine response to ND vaccine and MIT assay showed higher values for HCMI compared to LCMI lines. Among different divergent immune response lines, average Newcastle disease (ND) vaccine responses were higher in HSRBC and HCMI lines.

Random genomic differences between Red jungle fowl (RJF) and *desi* fowl were detected using RAPD, MASA and microsatellite markers. In general RJF showed less genetic similarity with other chicken breeds. A 448 bp fragment from 12S rRNA gene was amplified in RJF using universal primers and sequenced. It showed 99% homology with chicken sequences whereas 91% with other avian species including Green jungle fowl.

A resource population was developed for MHC characterization in guinea fowl. A 234 bp fragment and one 277 bp fragment was amplified using 2 separate sets of chicken specific primers. From AJ 2.1 clone, a 700 bp fragment having exon 2, intron 2 and exon 3 of BL ßII gene was eluted and biotin labeled is to be used as probe.

# Ex-situ conservation

Frozen semen doses of cattle (Kangayam, Punganur, Hariana, Amritmahal, Tharparkar, Dangi, Sahiwal, Gir), buffalo (Bhadawari, Tarai, Surti, Murrah), goat (Chegu, Black Bengal), sheep (Garole) and camel (Jaisalmeri) are being maintained in genebank for posterity.

# **Network Project on Animal Genetic Resources**

**Khillar:** The Khillar cattle are distributed in Satara, Sangali and Solapur districts of Maharashtra, and 48.9% farmers reared Khillar cattle. Average number of cattle was 2.85 per household. Most of farmers fed unchaffed green (98%) and dry (96%) fodder. Farmers (42%) used groundnut-cake, cottonseed-cake, and ready-made feeds available in market.

**Gangatiri:** The Gangatiri cattle are distributed in Varanasi, Chandauli, Ghazipur and Ballia districts of Uttar Pradesh and Bhabhua (Kaimoor), Buxar, Arrah and Chhapra districts of Bihar. Majority of the livestock owners (34.1%) have large land holding (>7.5 acres) followed by marginal (30.5%) and landless (19.5%). Only 16.4% of the cattle owners grow fodder. The general practice is to house the animals during night (86.81%) and allow them to graze during day.

Surti: In Kheda, Vadodra and Bharuch districts Surti buffaloes





Gangatiri cattle



Bonpala sheep

were 20.6, 30 and 56.1% of total livestock population, respectively. Majority of the buffalo owners (82 to 97%) were landless or small land holders having up to 5 acres of land. The Bharuch district has more tribal population. More than 95% of females and 70 to 80% of males are engaged in dairy business. The animal sheds are generally *kutcha* and open-walled. Breeding through AI is more common in Kheda and Vadodra districts.

**Bonpala:** Banpala sheep population estimate of nearly 1,671 was far less compared to the census figure of 5,530 sheep (Livestock census, Sikkim, 2004-05). The flocks remain healthy even without any vaccination or deworming, probably due to isolation and agro-climatic conditions. Common ailments include diarrhoea and minor wounds. The ewe can sustain only one lamb because of low milk production. They are sheared twice a year. The ban on



Chhotanagpuri sheep



Coimbatore sheep

forest grazing is forcing the sheep-owners to adopt the alternative sources of livelihood. It may cause a serious threat to Bonpala sheep and also to the yak and Siri cattle of Sikkim.

**Chhotanagpuri:** These sheep are maintained primarily for mutton and the wool, which is coarse, open and hairy. They are maintained on extensive grazing without any supplementary feeding. Chhotanagpuri sheep are of small size. with black, brown or white coat colour. Males are horned and females are polled. The body is covered with coarse and open fleece. Head, belly and legs are devoid of wool. Lambing rate is about 120–140%. Wool production ranges from 400 to 600 g/adult/annum. Most animals suffered with coccidiosis, amphistomiasis, trichuris and bursate. The incidence of amphistoms was least in Ranchi district and highest in West-Singhbhum.

*Coimbtore:* These sheep are reared mainly by Kurumba gounder, a shepherd community. Most flocks are migratory and some are stationary. The animals are maintained on grazing without any supplementation. Sheep are penned in harvested fields. They are medium size with black or brown head and white body. In many





Rampur Busbair sheep

animals the colour extends up to the shoulder. Some animals with black or brown spots in the head were also recorded. Ears are medium size and horizontally oriented. Rams are both horned and polled while ewes are hornless. The fleece is generally white, coarse and open. Most flocks practise selective breeding. The breed is primarily maintained for mutton and there is little income from wool.

**Mandya:** These sheep have roman nose, hanging wattles, light brown head and neck, dwarf and stocky body, well projected shoulder and chest cavity with well sprung up thoracic ribs, and uniform top-line. Evenly placed short and stumpy legs and wide apart hipbones indicated a square type meaty conformation of the breed. Sheep are mainly kept on grazing, however, a few farmers keep the animals on stall-feeding. Some of the farmers practise sheep tethering in their cropland, shifting from place to place and bringing back to their homestead in the evening.

Rampur Bushair: The breeding tract of Rampur Bushair sheep falls under varied climate from cold desert Himalaya to low lying Shivalik hills. These are primarily reared for wool and mutton purposes, and are also used as pack animals. Animals are predominantly brown followed by brown/white and white. The majority of animals are horned. The horns are curved. Average flock size ranged between 20-1,000 in migratory flocks and 2-20 in stationary flocks. Migration may take place from the permanent or temporary abode in the valley to the alpine pastures during summer only and to the foothills and plains in the winter. The flocks are primarily grazed on alpine pasture during summer, and on the harvested fields, forest areas, uncultivated fallow and barren lands during winter. Tree leaves and pods of fodder trees constitute an important feed resource during winter and early spring. The wool is coarse/carpet type. The lamb wool is fine and is used to manufacture tweeds of which, especially brown fetches higher price.

**Ganjam:** The Gola community rears Ganjam goats. The goats are reared in range system and stay in jungles throughout



Ganjam goat



Mehsana goat

the year. They solely depend on natural vegetation and no supplementary feed is given. Flock size ranges between 700–1,000. The male kids (9–12 months-old) and aged females are sold at regular intervals. Kids up to 3 months are allowed to suckle the mother. Animals are black and brown however, admixture of white, brown and black are also seen. The major source of income is from sale of male kids (9–12 months), milk and ghee and the manuring of the farmers' fields.

**Mehsana:** This goat breed is found in Banaskantha, Patan and Mehsana districts, its adjoining areas, and is mainly reared by *Rabaries* and *Vaghris* communities for milk and meat. Mehsana goats constituted 35.2% of total goat population in these districts. The male and female ratio is 1 : 17. Mehsana goats are mostly maintained on zero input under extensive production system. The farmers adopted both open (49.0%) and closed (11.7%) types of housing, and practise goat breeding in summer (May–July) only.









Rathi cattle





Kangayam cattle

Pandharpuri buffalo

## Genetic characterization

**Ponwar cattle:** Analysis of DNA with a battery of microsatellite markers revealed polymorphism at all microsatellite loci. Observed and effective number of alleles varied from 3 to 10 and 1.8 to 6.2, respectively. Mean number of observed and effective alleles was  $5.9\pm1.7$  and  $3.4\pm1.2$ , respectively. Observed and expected heterozygosity ranged from 0.194 to 0.8, and from 0.45 to 0.85 with the mean of  $0.497\pm0.176$  and  $0.682\pm0.113$ , respectively. The results reflect substantial genetic variability in Ponwar cattle.

**Kherigarh cattle:** All microsatellite loci were polymorphic. Observed and effective number of alleles varied. Mean number of observed and effective alleles was  $6.2\pm1.7$  and  $3.7\pm1.2$ , respectively. Observed and expected heterozygosity ranged from 0.260 to 0.808, and from 0.49 to 0.86 with the mean of  $0.574\pm0.131$  and  $0.717\pm0.090$ , respectively. The average observed hetrozygosity was lesser than the expected. The average expected gene diversity (Nei 1973) within the population ranged from 0.489 to 0.845 with an overall mean of  $0.709\pm0.090$ . Bottleneck test indicated that the population had not undergone bottleneck at least in the recent past.

# In-situ conservation

**Tharparkar cattle:** Elite Tharparkar cattle (73) were selected in Jaisalmer district. Bull calves (24) of more than 1 year of age were registered. The selection criteria were true-to-the breed characteristics, good health and dam's yield.



Tharparkar cattle

**Toda buffalo:** Their calves (20) were purchased and maintained at the University farm. The young bulls were given training on semen collection.

**Magra:** The selected ewes were reared under feeding and management practices. Elite ram-lambs (54) were selected on the basis of yearling body weight, dam's wool yield, wool yield of the individual, general health status and overall performance.

*Spiti horse:* Elite female animals (58) were selected from different villages. Out of the total male progeny born, 6 were selected and registered.

# Ex-situ conservation

Male calves and bulls of Nagori, Rathi, Kangyam cattle and Pandharpuri buffaloes were purchased. They were vaccinated against common diseases and trained for semen donation.

- Farm and year of calving, affect age at first calving, highest milk yield 300 days, peak yield etc. in Frieswal cattle
- Under field progeny testing programme the decreasing trend in age of calving of Frieswal daughters born from first to fourth set of bulls was observed
- Wet average, herd average and 300 day milk yield in Nili Ravi buffalo showed improvement
- Higher twinning was observed in Marwari sheep
- Jamunapari goat had 39.9% multiple birth with 1.42% kidding rate
  Barbari goats showed marked increase in body weight at 12
- months, milk yield at 90 days and kidding rate
- Pigs having lean meat with 60–70% less fat compared to Large White Yorkshire breed, were produced
- Egg production up to 40 weeks of age was highest in CARI-Sonali
- Age at sexual maturity declined in desirable direction in poultry
- Body weight of broiler at 5 weeks of age increased over previous generations
- Shank length and antibody titre showed improvement in Vanraja bird
- Improved varieties of fowls were evaluated in different localities

#### Cattle

*Frieswal:* The total population of Frieswal females at 43 Military Farms of the country was 17,099 (860 adult cows, 5,850 young stock and 1,389 calves).



The overall mean of milk yield 300 days was 3,038.7 kg. The effects of farm, parity, season and year of calving and age at first calving (AFC) were significant on milk yield in 300 days (MY300 days). Frieswal cows at Mhow produced (3,884.3 kg) the highest MY300. The average MY300 in first lactation was 2,622.1 kg. The cows attained the highest MY300 in sixth lactation (3,177.4 kg). Peak yield (PY) averaged 14.82 kg, and farm, parity, season and year of calving and AFC affected it significantly. Average lactation length (LL) was 319.1 days, and farm, parity and season and year of calving and AFC affected it significantly. The overall mean of AFC was 990.0 days, and farm, season and year of birth affected it significantly. The simple average of weight at first calving (WFC) was 375.7 kg, and farms, season and year of birth, and AFC affected it significantly.

# Indigenous breeds

**Hariana:** The female herd strength was 1,176. The breeding population contained 808 female and 12 breeding bulls with the overall conception rate of 61%. The per cent cows in milk, wet average per day per cow and herd average per day were 39.10%, 4.24 kg and 1.73 kg, respectively. Young bulls put to training took on an average  $35.88 \pm 1.78$  days to be ready for draught purposes. The mean fatigue score for empty cart ranged between 2.20 and 2.90 after 2 hr work and 2.75 and 3.15 after 3 hr work. In cart with 8 q load, the corresponding values were 3.10 and 3.60 and 3.90 and 4.35, respectively. Average carting ability (as per C.K. Thomas method) was 18.34.

**Ongole:** The female herd strength was 1,360. The breeding population comprised 914 females and 5 breeding bulls The conception rate was the highest (66.81%) at associated herd, Lam, followed by at GP Unit, Lam (56.90%), and was the lowest at associated herd, Chintaldevi (33.60%). The per cent cows in milk, wet average per day per cow and herd average per day were 35.66%, 3.18 kg and 1.13 kg, respectively. The age at first calving averaged 50.08 months. The average lactation milk yield and peak yield was 402.6 and 2.59 kg. The overall average of service period and lactation length were 254.4 and 181.5 days, respectively. Their superiority over the herd average ranged between 18.71 and 29.41 kg (3.47 to 5.45%). Draught studies were undertaken on 10 animals by using single harness plough with digital dynamometer. Draught power varied from 0.68 to 0.86 hp among the bulls.

# Field progeny testing

The genetic improvement through field progeny testing of Frieswal cattle is being carried out at three units.

**PAU unit:** So far 81 bulls have been used in 4 different batches. During this year semen from fifth set of 22 crossbred bulls was used, and 5,530 inseminations were carried out. The conception rate was 40.5% and 2,063 pregnancies were confirmed. Daughters (624) from first 4 sets have completed their first lactation (305 days) with average yield of 2,697.8 (2,254 to 3,001 kg), 2,827 (2,397 to 3,265 kg), 2,829 (1,710 to 3,406 kg) and 2,905 (2,111 to 3,509 kg) kg, respectively, indicating considerable variation in the breeding values of the bulls used in these sets.



Frieswal cow—Milk yield of a cow at Mhow was recorded as 3.884.3 kg in 300 days



The average fat percentage of daughters of first, second, third and fourth set of bulls was 3.7, 3.8, 3.9 and 3.9 respectively. The average age at first calving of daughters born from four sets of bulls was 1,192, 1,145, 1,117 and 981 days respectively.

**KAU unit:** The overall conception rate in 5 batchs was 32%, and 401 female calves have been born. Daughters (759) from first 4 sets of bulls have completed their first lactation (305 days) record and the average yield observed was 2,079, 2,903, 2,074 and 2,226 kg respectively, indicating the superiority of the progeny over the other animals in the field born from other sources. The average age at first calving of daughters born from first, second, third and fourth set of bulls was 1,110, 1,024, 1,280 and 1,031.3 days respectively. The average fat percentage in the second, fifth and eighth month of lactation was of 3.8, 3.9 and 4.0%.

**BAIF unit** During the year 4,751 inseminations were performed, 3,806 inseminations were followed for pregnancy diagnosis and 1,631 pregnancies were confirmed. The average conception rate of this batch was 42.85%. Out of 20 sires under test, 4 sires recorded conception rate above 45%, and of 10 ranged from 41 to 45%. The average age at first calving was 32.29 months with average lactation yield of 2,748 kg.

# **Buffalo**

The Murrah is the most important milch buffalo breed with its home tract in central parts of Haryana. At the CIRB, Hisar, a herd of Murrah buffaloes is being maintained for progeny testing programme and to make available progeny tested superior bulls for buffalo farmers of the country. The wet average of Murrah herd at the institute was 6.30 kg while herd average was 4.65 kg.



# Network Project on Buffalo Improvement

Under this project, the ninth set of breeding bulls – originating from nominated matings with elite females – is being tested currently. In addition, field progeny testing has also been initiated to increase accuracy of selection. The top ranking bull in the institute herd in the second set showed sire index of 1,987.4 kg. Doses of frozen semen from progeny tested bulls are available at various centres under the project, and total stock of frozen semen from Murrah bulls at the institute is over 285,000.

Progeny testing programme for Nili Ravi breed is being undertaken at Sub Campus of the institute at Nabha. The wet average, herd average and 300 day milk yields were 6.86, 4.65 and 1,848 kg, respectively, with improvement of 17.1, 35.6 and 26.0% as compared to values in 1992–93. Similarly, age at first calving and calving interval also showed significant improvements – over 10% each.

Elite herds of Jaffarabadi, Surti, Bhadawari, Pandharpuri, Godavari and Swamp buffaloes have also been established at the respective centres under Network Project. Average 305 days or less lactation yield in Jaffarabadi buffaloes was recorded as 2,069, while in Pandharpuri 1,687 kg, Surti 1,633 kg, Godavari 2,540 kg, Bhadawari 1,029 kg and in swamp buffaloes 496 kg.

# Sheep

# Sheep for fine wool

*Carpet wool:* The Magra breed is considered to be the best indigenous carpet wool producing sheep. Their body weight at birth, 3, 6, 9 and 12 month were 3.10, 16.95, 22.68, 26.32 and 28.37 kg, respectively. The tupping, lambing on available and



Hariana cow and bull



tupped basis were 97.6, 69.5 and 71.2%. Fibre diametre, hetro fibres, medullation percentage, staple length and crimp showed an increase.

At the Arid Region Campus Bikaner, Marwari sheep - a breed, which is preferred by migratory farmers for its hardiness - showed weight at birth, 3, 6, 9 and 12 month as 3.24, 16.30, 22.00, 26.46 and 28.19 kg, respectively.

**Dual purpose sheep:** Genetic improvement in Bharat Merino were aimed to develop it as a dual purpose (mutton and wool) sheep. At Avikanagar, body weights at birth, 3, 6, and 12 months of age were 3.11, 14.79, 22.62 and 27.23 kg respectively. The overall survivability was 90.12%. Culling was 13.92%. The tupping and lambing percentage on the basis of ewes available were 91.18 and 80.64. Tupping% was 88. Overall mortality was 7.06%. The average fibre diameter was 23.24  $\mu$ , medullation was lesser than 1%, and the average staple length was 8.1 cm in the annual clip. At North temperate Research Station, Garsa, Himachal Pradesh, the body weighs at 3, 6, 9 and 12 months of age were 12.15,19.62, 21.53 and 25.09 kg respectively.

#### Goat

*Genetic improvement in goats in field and farm conditions:* Genetic improvement of Jamunapari 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 dam) indicated improvement in both milk yield and body weight. The mean body weights at birth, 3, 6, 9 and 12 months of age were  $3.37\pm0.03$ ,  $11.59\pm0.10$ ,  $16.49\pm0.22$ ,  $22.42\pm0.29$  and  $28.44\pm0.33$  kg, respectively. The improvement in body weight at 9 and 12 months of age were 38.22 and 39.96% more over the years. The kids under feedlot weighed  $21.84\pm0.43$ ,  $32.67\pm0.53$  and  $38.88\pm0.44$  kg at 6, 9 and 12 months of age, respectively. Improvement in 90 and 140 days milk yield was recorded as 88.85 and 82.0%, respectively, over the years. This breed is prolific as apparent from 39.9% multiple births with 1.42 kidding rate. The heritability estimates for body weight at birth, 3, 6, 9 and 12 months of age were  $0.37\pm0.09$ ,  $0.24\pm0.08$ ,  $0.18\pm0.07$ ,  $0.23\pm0.08$  and  $0.26\pm0.08$ , respectively. Genetic and phenotypic correlations among adjacent body weights were high.

Similar approach for improvement of Barbari breed yielded encouraging results. Body weight at 12 months has recorded about 18.46% increase in growth rate over the years. The milk yield at 90 days showed about 63.96% increase over the years. The kidding rate was about 1.61 indicating highest population growths in Barbari goats. Study conducted on Jakhrana goats, a milch breed of Rajasthan revealed 37.21% multiple birth. Body weights of adult male and female were 43.84 and 68.72 kg, respectively. And 140 days milk production was recorded as 81.72 kg.

A field study, undertaken in the home tract of Zalawadi goats

### Jamunapari goats

The home tract of Jamunapari goats, the Chakarnagar area (Chambal ravine) of Uttar Pradesh, is known for its natural biodiversity. Improvement programme is being carried out by the CIRG in 2 adopted villages. The observation regarding population dynamics, growth parameter, milk yield, kidding pattern, disease occurrence have been recorded. The socio-economic and parasitic characterization of field condition indicated that goat rearing was an integral part of the livelihood of locals. Body weight, milk yield, and reproductive performance were recorded. The milk production in 90 days of lactation was 105.05 kg and body weight at 9 month of age was 25.40±0.36 kg, which indicated increasing trend over the years.



Jamunapari goat at field conditions





Jamunapari goat

in Surendra Nagar district of Gujarat, indicated that goats were mostly reared by *Robanes* and *Bharwal* (nomadic tribes) communities locally called *Maldhary* under extensive system of management. Zalawadi is a large size, black colour goat with spiral horn. The average flock size ranged from 13 to 71. The peak milk yield was observed as 2.25 kg and lactation length ranged from 5 to 7 months. Ratio of buck to adult doe was 1:80. This breed is prolific. Farmers were suggested to select bucks considering breed phenotypes, height, weight and dam's milk yield, and does on the basis of breed phenotype, milk yield, udder size and teat length and height.

#### Rabbit

At the NTRS, Garsa, the German Angora rabbits were being improved. The average annual wool yield of general and breeding flocks was 817.27 and 928.92 g, respectively. The respective performance for GA progeny flock is 483.5 g. Females had shown a general tendency to yield higher wool as compared to their respective male counterparts. The average annual wool yield of adult general flocks of BA, RA and A1-cross are 495.83, 454.39 and 423.39 g, respectively. At the SRRC, Mannavanur, body weight at 12 weeks was 1.741 and 1.776 kg in WG and SC, respectively.

# Pig

Besides developing infrastructural base for the NRC on Pigs, a base line survey was carried out to document the existing status of pig production in the 8 states of North East including Sikkim.

All India Coordinated Research Project on Pigs: Crossbred pigs were produced at centres of the AICRP on Pigs, with increasing level of exotic inheritance up to 87.5%. At the Assam Agricultural University, Khanapara, and IVRI campus, Izatnagar, production of crossbreds (indigenous  $\times$  landrace) was continued, and at Jabalpur, Tirupati, Katupakkam, Mannuthy and Goa centres crossbreds between indigenous and Large White Yorkshire were produced. At the Mannuthy centre, Kerala Duroc breed was used as the terminal sire over the crossbred between indigenous and Large White Yorkshire (75%) so as to produce lean meat with 60– 70% less fat compared to LWY breed. All the centres resorted to *inter-se* mating either with 75% or with 87.5% genetic groups to study the stability level.

# Camel

The overall calving was 92.86%, with Bikaneri 91.67%, Jaisalmeri 90% and Kachchhi 100%. The conception rate was 85.11% which is better than the previous five years performance. The continuous selection, change in mating system and procurement of good males in time from the breeding tract brought this improvement. The lowest age at first conception was observed in Kachchhi (1,728.88±151.07 days) followed by Bikaneri (1,764.57±70.25 days) and Jaisalmeri (1,897.07±110.33 days). A large SE in Jaisalmeri and Kachchhi suggests the scope for improvement. The age at first calving was 2,184.48±83.22 days. The average of first gestation period was 393.64 days and that of subsequent gestations 385.88 days.

# Poultry

# **Poultry for egg**

In layers, significant genetic responses for 40 weeks of egg production was observed as  $0.99\pm0.18$  and  $0.75\pm0.19$  eggs per generation in IWH and IWI strains, respectively. Among crossbreds, the egg production up to 40 weeks of age was highest (108.72 eggs) in CARI-Sonali followed by 104.5 eggs in CARI-Priya and 80.73 eggs in CARI-Debendra. The mean egg weight at 40th week of age ranged from 51.9 to 55.1 g in different lines. The fertility in RIR stocks ranged from 88.1 to 91.9%, and hatchability ranged from 64.8 to 73.5%. Genetic responses of  $1.02\pm0.19$  eggs and  $63.9\pm10.8$  g of egg mass per generation at 40th week were highly significant.

# **Poultry for meat**

In broiler, overall average body weights at 3 and 5 weeks in colour synthetic male line (CSML) were  $567.86\pm1.64$  and  $1,054.65\pm3.40$  g, respectively. The estimates of heritability for 3- and 5-week body weight were  $0.19\pm0.04$  and  $0.24\pm0.05$ , respectively, in CSML. The genetic and phenotypic response for 5-week body weight in CSML and synthetic male line (SML) were  $24.64\pm4.66$  and  $36.81\pm6.56$  g per generation, respectively. Fertility percentages were 78.3 and 81.0 in colour synthetic female line (CSFL) and synthetic dam line (SDL), respectively. The corresponding percentages of hatchability (FES) were 89.0 and 83.1. The overall average of body weight at 3 and 5 weeks in CSFL were 538.50\pm1.48 and





A pair of colour broiler line

1,040.48±3.05 g, respectively. The corresponding means in SDL were 482.57±3.89 and 1,072.39±5.07 g, respectively. The estimates of heritability for 3-and 5-week body weights were  $0.16\pm0.03$  and  $0.21\pm0.03$  for CSFL, and  $0.18\pm0.04$  and  $0.26\pm0.05$  for SDL. Genetic and phenotypic responses for 5-week body weight in CSFL line were 27.87±3.01 and 40.05±5.58 g/generation, respectively. Fertility percentage in IC3, IR3 and CARIBRO-Tropicana were 76.2, 89.1 and 68.7, respectively. The mean body weights at 3 and 5 weeks of age in CARIBRO-Tropicana, IC3 and IR3 were 449.10±3.44 and 873.46±6.39 g, 293.65±4.10 and 614.35±6.32 g, and 367.76±4.14 and 709.27±8.03 g, respectively.

# All India Coordinated Research Project on Poultry Breeding

# **Poultry for eggs**

Under the AICRP on Poultry Breeding, 6 layer strains of White Leghorn were subjected to selective breeding through intrapopulation selection for egg production up to 64 weeks of age, superimposed with independent culling level for egg weight at 28



Vanaraja male line parents

# Management of costal agro-eco system affected by super cyclone in Orissa

The Regional Centre of CARI has been allotted an NATP research project under the costal agro ecosystem for rehabilitatation of the super cyclone affected families by introducing poultry as a source of income generation. The high yielding birds developed at the CARI were distributed among 2,300 farm families and ducks among 400 families.

The dual-purpose bird CARI-Devendra developed by crossing between synthetic colour broiler as male and RIR as female line were distributed in the costal super cyclone affected families in 13 villages. Similarly Gram Priya and Vanaraja chicks were also distributed. The centre with full technical support monitors the performance of these crosses. Most of the affected families earned Rs 1,000–2,000 either by sale of eggs or as meat bird or chicks.

- The rapid growth of this bird helped the poor schedule cast families in the rehabilitation and regeneration of their lost livestock.
- The growth performance and production of these crosses are far better than the native birds available in these areas.
- The work and achievement of the NATP project is very encouraging as the adapted farmers are able to regenerate their own stock using these high yielding birds with the help of native hens for regular income generation.

weeks of age and layer house viability. At the KAU, Mannuthy, the part period egg production of IWN and IWP strains ( $S_{21}$  generation) up to 40 and 64 weeks of age increased over previous generation. The 40-week egg weight in both the strains improved marginally over preceding generation. The fertility and hatchability in IWN improved in the present generation. At the AAU, Anand, the egg production up to 64 and 72 weeks and egg mass production up to 72 weeks of age in IWN and IWP strains, showed improvement. The fertility and hatchability in IWN and IWP strains, showed improvement. The fertility and hatchability in IWH, IWI and control population ( $S_{27}$  generation) at the CARI, Izatnagar, improved over the preceding generation. The egg production up to 40 weeks of age increased in IWH (by 4 eggs) and IWI (by 11 eggs) over previous generation.



Vanaraja female line parents



#### SUCCESS STORY

#### Household poultry farming in Manipur

A newspaper hawker, Shri Shyam Kumar Singh of Shamushang village, Imphal West District maintained a small Vanaraja unit. Initially, he started with 20 birds (5 males and 15 females). The unit was improved to retain 90-100 adult hens with 20-25 cockerels. His annual income was Rs 18,000 from his primary profession of newspaper sales. His family has his mother, two children and wife. He made low cost shelter and feed for Vanaraja birds. In 2004, he disposed 15 cockerels and earned Rs 3,035. He



Vanaraja rearing in Manipur —a joy for whole family

regenerated 273 straight run chicks, and the males weighed 2.44 kg and the females 1.76 kg, at 17 weeks of age. The process of multiplication and disposal of birds continued and he earned Rs 9,427. He is being seen as an achiever of rural poultry farming in the region.

The age at sexual maturity declined in desirable direction on genetic scale to the tune of 0.58 and 0.37 days in IWH and IWI strains, respectively. The egg production up to 40 weeks of age in HI strain cross (104.5 eggs) increased over the preceding generation at this centre. The fertility and hatchability in IWD ( $S_{25}$ ) and IWF ( $S_{24}$ ) lines at the ANGRAU, Hyderabad, improved over previous generation. The egg production up to 40 weeks of age in IWD (115 eggs) and in IWF (117 eggs) improved over the last generation. The phenotypic response (for 5 generations) for egg production up to 64 weeks of age (primary trait) in IWD was 5.83 and in IWF 4.91. The genetic response (for 5 generations) of this trait in IWD was 2.40, and in IWF 1.48.

# **Random Sample Poultry Performance Test**

The IBL-80 of the PAU, performed best with 6-week-body weight of 1,540 g and of 7-week body weight 1,972 g; corresponding feed conversion efficiencies of 2.19 and 2.26, respectively, and dressing percentage 74.7%, at Random Sample Poultry Performance Test, Gurgaon.

Pureline White Leghorn populations, viz. IWH, IWI and IWK, are being maintained and selected at the PD on Poultry, Hyderabad, for part period egg production to 64 weeks of age following Osborne Index with independent culling level selection for egg weight at 28 weeks of age. The IWK and IWI lines produced 3.3 and 7.3 more eggs to 40 weeks of age over previous generation. The egg weight at 40 weeks of age for IWH and IWK lines increased by 0.7 and 1.0 g over the preceding generation, respectively. The HI (IWH  $\times$  IWI) cross was superior to IH (IWI  $\times$  IWH) cross and laid 270 eggs up to 72 weeks of age.

# **Poultry for meat**

This component of AICRP on Poultry Breeding included colour synthetic broiler lines (CSML) and CSFL and corresponding control at CARI, Izatnagar; SDL at OUAT, Bhubaneshwar; PB-2 at PAU, Ludhiana, and UAS, Bangalore. Mass selection for 5-week body weight with due weightage for conformation traits in the male line and 5-week body weight, egg production and hatchability in the female lines have been continued to achieve the set target in the meat stocks. The mean body weight at 5 weeks of age in PB-2 and control lines in S<sub>10</sub> generation at the UAS, Bangalore, was 1,003 g and 853 g, respectively. Feed conversion ratio up to 5 weeks of age was 2.01 in PB-2 and 2.04 in control line. The body weight at 5 weeks of age in PB-2 and control lines in the present generation was 824 and 703 g, respectively, at the PAU, Ludhiana centre. The corresponding feed



Gramapriya chicken scavanging in rural backyard



A pair of Dahlem-Red line



# Free-range household poultry farming in NEH region

The activity of free-range poultry farming with low input costs was initiated in Manipur, Mizoram, Nagaland and Arunachal Pradesh under NATP, and its impact over 5 years was evaluated. PD on Poultry supplied fertile eggs and day-old chicks of Vanaraja germplasm to the centres located in these states.

In Manipur, the adult females weighed 2,190 and 2,800 g at 20 and 40 weeks of age, respectively. They matured between 164 and 178 days of age, and on an average produced 2.8 eggs/week. A sample study conducted in Manipur over 64 beneficiaries in 18 locations showed that 440 surplus adults were sold for meat purposes and earned Rs 76,010 @ Rs173/bird. Also, the beneficiaries multiplied chicks on their own. Similarly, in Nagaland 337 families reared and multiplied Vanaraja birds for domestic purpose. A perceptible increase in egg and poultry meat consumption was noticed after the introduction of family poultry farming in the pockets of Nagaland. In Arunachal Pradesh, the adult females matured between 172 and 187 days of age and produced 3.3 eggs/ week/bird. Surplus males and females were disposed to earn supplementary income of Rs 86,662 @ Rs 224/bird. Similar trend was also witnessed in Mizoram.

efficiency was 2.18 and 2.56 in PB-2 and control lines, respectively.

The genetic and phenotypic responses for 5-week body weight were positive and significant for SDL population at the OUAT centre. The feed conversion ratio up to 5 weeks of age was 1.94 in this population (SDL) in  $S_{10}$  generation. The body weight at 5 weeks of age in CSML (1,054.65) and CSFL (1,040 g) in the present generation was improved by 16.7 g and 20.5 g, respectively. The genetic and phenotypic responses for 5-week body weight (for last 4 generations) were 24.6 and 36.8 g in CSML and 27.9 and 40.1 g in CSFL lines, respectively, in  $C_4$  generation at the CARI, Izatnagar centre. The development of purebred dwarf dam line was undertaken at the JNKVV, Jabalpur. The dwarfing gene line



Krishibro-multicolour broiler chicks

was subjected to mass selection for 6-week body weight. Also, egg production and hatchability were considered for improvement in this population. A colour dwarf line was also maintained at this centre. An improvement of 22 g was observed in 6-week body weight in the white dwarf line during the  $G_6$  generation. An improvement of 1.9 and 1.0 eggs in white and colour dwarf lines, respectively, was observed over previous generation.

#### **Rural poultry production**

**Development of lines for production of germplasm** suitable for rural and tribal areas: A colour line was used at the PD on Poultry, to develop male line for production of Vanaraja, a dual-purpose germplasm for rural poultry production. Selection was practiced for shank length and antibody titre, the desired traits. In S<sub>6</sub> generation, age at sexual maturity, body weight at 20 and 40 weeks of age, egg weight at 28, 32 and 40 weeks and egg production up to 40 weeks of age were 172.5 days, 2,134 g, 2,734 g, 49.8 g, 52.6 g, 54.3 g and 43 eggs, respectively.

A multi-colour plumage population is being improved as female line of Vanaraja. In S<sub>4</sub> generation of female line, the age at sexual maturity, body weight at 20 and 40 weeks of age, egg weight at 32 and 40 weeks and egg production up to 40 weeks of age were 153.9 days, 2,306 g, 2,543 g, 52.3 g, 53 g and 61.1 eggs, respectively. During juvenile stage, due weightage was given for antibody titre, shank length and plumage colour. During S<sub>5</sub> generation, body weight at 4 and 6 weeks of age were 382 and 727 g, respectively. Shank length and antibody titre showed an improvement of 0.5 mm and 0.16 (log<sub>2</sub> value), respectively, over the preceding generation, which was desirable to ensure better survivability in the harsh environment of rural areas. The average intensity of selection for shank length and antibody titre was 0.26 and 0.73  $\sigma$ . The heritability was low to moderate for body weight at 4 and 6 weeks of age and low for shank length and antibody titre.

*Tinted egg layer for backyard poultry farming:* Dahlem Red line was utilized as female line for production of Gramapriya, which is a layer variety developed by the PD on Poultry, for backyard poultry production. It has completed 3 generations of selection for part period egg mass. In  $S_3$  generation, age at sexual maturity, body weight at 20 and 40 weeks of age, egg weight at 28, 32 and 40 weeks of age and egg number up to 40 weeks of age were 178 d, 1,176 g, 1,855 g, 51.3 g, 53.9 g, 56.1 g and 79.6 eggs, respectively.

**Rural poultry germplasm evaluated at Agartala:** At the ICAR Research Complex for NEH Region, Agartala, testing of various germplasm developed for augmenting rural poultry production in the region under AICRP on Poultry Breeding, was continued. Krishna J, developed at the JNKVV, Jabalpur, and Gramapriya, developed at PD on Poultry, Hyderabad, were evaluated.



Fertile eggs of Gramapriya and Krishna J were hatched and reared at the Institute farm up to 12 weeks of age. The body weight of Krishna J at Institute's farm was higher than that at under farmers' field. Body weight of Gramapriya was higher compared to Krishna J under both the management systems.

**Backyard poultry:** In indigenous fowl, the highest fertility (81.0%) was observed for Aseel Kagar but hatchability (FES) was highest for CARI-Red (82.4%) among the purebreds. McNally, logistic curvilinear and Adams-Bell model were the best fit to describe egg production of Frizzle (F),  $F \times CR$  and  $CR \times F$ , respectively. For egg production curves of Kadakanath (Kn), Kn  $\times$  CR and CR  $\times$  Kn, the curvilinear logistic, monomolecular minus linear term, and logistic minus linear term, respectively, were best fit models with R<sup>2</sup> values of 91.6, 98.4 and 97.4%. The improved varieties of indigenous fowls developed for backyard poultry production were evaluated in different localities.

In general, the mission to promote free-range poultry farming at low input costs and an awareness of its utility for the household purpose was realized in these 4 NEH States. The families had access to eggs and chicken meat for consumption and for earning supplementary income through disposal of poultry produce.

# **Guinea** fowl

In guinea fowl, average body weights at 12 weeks of age were  $861.25\pm5.32$  g,  $839.65\pm6.72$  g and  $826.25\pm3.19$  g in Lavender, Pearl and White guinea fowl varieties, respectively.

# Quail

A new white plumaged phenotype of quail was developed for commercial exploitation. Homozygous (wh/wh) has white plumage with dark eye while heterozygous bears two-colour pattern known as Tuxedo.

# Foot-and-mouth disease

Field specimens (683) from different outbreaks in the country were examined and subjected to serotype confirmation by sandwich-ELISA using the direct materials. No virus could be detected in 317 sample, and from rest type O, A, and Asia 1 were detected. Outbreaks due to type O virus were recorded in 14 states, and types A and Asia 1 in 5 states. All the three serotypes were prevalent in Madhya Pradesh, West Bengal and Gujarat. Samples were processed for revival in cell culture (BHK-21) system and in 21 samples virus could be recovered comprising type O, Asia 1 and type A. The field isolates of type O and Asia 1 are antigenically related to the in-use vaccine strains in serological tests indicating that the vaccine strains are good enough to cover the circulating field isolates. However, in type A the field situation demands change of

- Immune response was assessed in sheep for early diagnosis of haemonchosis
- Suitable drug delivery system has to be developed to protect the allelochemicals of herb Lawsonia inermis
- Babesia equi-specific ELISA was developed
- Abortus bang ring test was found reliable to test brucellosis in mithun
- Matrix gene of Indian isolate of avian influenza virus (H9N2) was sequenced
- Several herbal medicines were tested
- Sensitive diagnostic techniques and effective vaccines were developed for IBD, rabies, turkey-pox, Ranikhet disease etc.
- In FMD type A the field situation demands a change in vaccine strains
- Complete nucleotide sequence of several Asia 1 field isolates were determined
- · Indigenous staining technique for leptospira was developed

vaccine strains. Work in this direction indicates that IND 81/00 (reference strain of genotype VII) could be a candidate.

Molecular epidemiological analysis of type O virus indicates the hide- and-seek nature of Pan-Asia strains in the disease outbreaks. The type A field isolates had a deletion at VP359 and formed a separate cluster in the genotype VII. This novel genetic cluster was observed as early as November 2002 from the state of Assam (IND 24/03) and within 1 year it spread into 5 other states including Gujarat at the western and Karnataka at the Southern parts of India. In type Asia 1, the newly emerged viruses that were responsible for disease outbreaks in different states last year were involved in all the outbreaks of this year also, indicating the dominance of divergent group of virus. The complete nucleotide sequence of several type Asia 1 field isolates was determined. The tree topology of the individual genes was similar to that of the complete coding region or primary polyprotein cleavage products in isolation when used for an equivalent analysis. The identification of residues, positive selection of some of which are antigenically critical, made a beginning in the understanding of antigenic features of this serotype. The virus repository of the Project Directorate on FMD has 1,193 (O, Asia 1, A, C) field isolates.A multiplex-PCR (mPCR) for serotyping of FMD clinical samples developed earlier was further evaluated on clinical samples, and now it can be used as a backup test for ELISA for FMDV serotyping. Work was initiated to evaluate the potentiality of the expressed proteins in comparison to the whole virus particle in sandwich-ELISA. The expressed VP1 protein of type O was specific.

#### Animal disease monitoring and surveillance

Computer interface based diagnostic kits were developed for diagnosis of brucellosis and IBR antibody as per the standards of IAEA.



### National Animal Disease Referral Expert System

National disease database for the past 15 years and meteorological and agro-ecological data from all the agroclimatic zones of the country were compiled, as a part of National weather based animal disease forecasting. And forecasting for 14 animal diseases was made with 75 to 98% accuracy.

The outputs from NADRES would provide the following:

- GIS based animal health information system
- The livestock disease forecasting system
- Demarcation of country into eco-pathozones based on specific livestock diseases
- Livestock disease economics

National sero-epidemiological surveys for brucellosis and infectious bovine rhinotracheitis, showed an overall incidence of 18 and 51% respectively. Sero-epidemiological surveys in bluetongue and PPR were conducted based on stratified sampling framework, and their incidence showed an increasing trend. The sero-prevalence of bluetongue is to the extent of 51% and that of PPR 15.3%, and a further screening is under progress.

Isolates (599) of different serovars of leptospira are being maintained. *Leptospira* from an apparently normal elephant was isolated and its typing is in progress. At the ADMAS an indigenous staining technique was developed for leptospires, which is very useful even at field level. The prototype kit is being validated at field laboratories.

#### SUCCESS STORY

#### **Effect of vitamin E supplementation**

Dairy animals generally develop mastitis immediately after parturition because of reduced immuno-competence. Supplementation of vitamin E reduceed the incidence of both clinical as well as sub clinical mastitis leading to significant improvement in milk yield during first month of lactation (20-30%). The oxidative stability of milk produced from vitamin E supplemented cows also improved as compared to their control counterparts. Milk vitamin E content of vitamin E-supplemented animals was also higher. Retention of foetal membrane and metritis were less in vitamin E supplemented cows and buffaloes. It is recommended that cows and buffaloes should be daily supplemented with vitamin E @ 1,000 IU/head and 1,500 IU/ head, respectively, from 30 days prepartum to 30 days postpartum. Daily dose of vitamin E supplementation for 60 days amounts to Rs 60 and 90 in cows and buffaloes, respectively. The technology is simple, cheap and cost effective, seeing the heavy losses (Rs 6,000 crores/annum) incurred due to mastitis in dairy animals, and needs to be propagated among dairy farmers.

Online official software was developed for animal disease monitoring and forecasting. The stratified random survey revealed prevalence of nationally important animal diseases like IBR (25%), brucellosis (16.31%), PPR (10.78%), bluetongue (37%), CCPP (34%), swine fever (33%) etc.

# **Gastro-intestinal parasitism**

Epizootiological studies revealed prevalence of GI nematodes infection in sheep, goat, cattle and buffalo in various districts of Uttaranchal, Himachal Pradesh, Uttar Pradesh, Haryana and Delhi. The prevalent infections were of *Haemonchus* sp., Trichostrongylus spp., Oesphajostomum spp., Bunostomum spp., and *Strongyloides* spp. in all the livestock, whereas *Cooperia* spp. in cattle and Ostertagia in sheep and goats were also recorded. In Rajasthan bioclimatograph was prepared for prediction of H.contortus and Trichostrongylus spp. infection in relation to meteorological factors. Software named FROGIN was developed for forecasting of haemonchosis in sheep for arid and semi arid zones. The software gives result as predicted faecal egg count (FEC) on start of the month, intensity of FEC for next 60 day and pasture level burden for the month which will be helpful to improve worm control scheme. In Tamil Nadu overall prevalence of GI nematode infection was 42.6% in sheep, goat, cattle, buffalo. In Madhya Pradesh and also in Chhattisgarh, epizootiological studies were conducted. Haemonchus spp. was predominant infection in cattle. In pigs prevalent infections were Ascaris suum, Strongyloides and Schistosoma incognitum. In the northern hills and plains of Chhattisgarh the strongyle infections were

#### SUCCESS STORY

#### Liquid phase blocking ELISA for detection of antibodies in vaccinated animals

The liquid phase blocking ELISA (LPB ELISA) test was developed at the Project Directorate on Foot-and-mouth Disease (PD FMD), Mukteswar. It is a sensitive and specific serological test for determining the protective antibody levels in animals following vaccination. The LPB ELISA helps in determining the protective antibody levels in animals following vaccination by comparing the pre- (0 day) and post-vaccinated (21st day) sera from animals. The results (expressed as log<sub>10</sub> antibody titre) are obtained within 24 hr and have replaced the more cumbersome serum neutralization test for determining the protective antibody levels in animals. A titre of log<sub>10</sub> 2.1 indicates protection against homologous challenge i.e., challenge with virus, which is used for making the vaccine. However, protection against heterologous strains (field strains) will depend on the antigenic similarity between the field and vaccine strains. The test is being used by the regional centers of the PD FMD for measuring post vaccinal immune response in animals for the FMD control program of Government of India.



#### **Patents**

- Development of a process of preparing a bio-organomineral formulation for the therapy of skin ailments in animals
- A process for preparing an indigenous drug formulation for the treatment of diarrhoea in animals
- Development of a technology for an area specific mineral mixture to increase productivity of bovines i.e. milk yield and body weight in Uttar Pradesh State

prevalent in cattle and buffaloes. In Meghalya the GI parasitism showed increasing trend in cattle and decreasing trend in pig. In ducks the infection recorded in Assam were *Ascaridia galli*, *Strongyloides* spp., *Capillaria* spp., *Echinostome* spp, *Hymenolepis* spp. and *Rallietina* spp. In Sikkim prevalence of GI helminths was significantly more in subtropical and high humid zones. During summer and autumn infection rate was higher in goats followed by cattle and lowest in yak. In West Bengal prevalence of GI parasitism was higher in small ruminants (71.98%) than that in large ruminants (46.13%).

For early diagnosis of haemonchosis in sheep immune response was assessed with different antigens using different tests. In Western blotting, third week experimental post infection (PI) sera recognized 26, 60 and 120 kDa polypeptides whereas with the naturally infected sera only 60 and 120 kDa polypeptides were recognized. In affinity purified fraction 60 and 120 kDa polypeptides were detected in SDS-PAGE. In immunoprecipitation 26, 32, 60 and 120 kDa polypeptides were detected in excretory-secretory antigen (ES) by antibodies as early as first week PI. In dot-ELISA solid dot was formed on the nitrocellulose membrane with B-1 fraction and the antibodies against H.contortus were detected as early as first week PI. In dip stick-ELISA antibodies were detected in the sera as early as first week PI. In western blotting 152 kDa in Oesophagostomum spp. and 31 kDa in Bunostomum spp. were immunodominant. Dot-ELISA was standardized for detecting infection in goats and cattle. Immunization trials were conducted by using con-A H11 antigen. In lamb reduction in worm count was 77.5%, whereas egg count reduction was 85.1%. In the immunized animals the abomasum did not show lesions. SDS-PAGE of the gut protein of Ascaris suum revealed polypeptides ranging between 26 to 118 kDa. With DID antibodies in

### Technologies commercialized and MoU signed

- Development of an indigenous methodology: IVRI crystoscope as a field tool for determining optimum time for fertile insemination in animals
- 2. MoU was signed between institute and 2 private firms



Equiherpes B-ELISA kit

hyperimmune serum raised against *Ascaris suum* gut protein showed precipitin line, which was also observed in countercurrent immuno-electrophoresis indicating antigenic nature of protein. Anthelmintic resistance with fenbendazole and levamisole was recorded at Government Sheep Farm, Dim Chandi, Uttaranchal. Nematophagous fungi such as *Arthrobotyrus oligospora* were having excellent predatory activity against *Haemonchus* spp. larvae and could survive in gut environment.

# Haemorrhagic septicaemia

Specimens, comprising 5 morbid materials, 56 sera samples and 154 cultures were received for isolation and confirmation of *Pasteurella*. Only 74 out of 154 cultures, were identified as *Pasteurella* on the basis of biochemical tests and PM-PCR assay. No isolation could be made from morbid material. A successful trial of low volume saponified HS vaccine was carried out in dairy cows. The animals were 100% protective at 9 months and 80% at 12 months of post vaccination against challenge using HS virulent culture. Combined vaccine prepared against HS and FMD was tested in calves. The animals were protective against both the antigens at 28 days of post vaccination on challenge test.

Molecular studies were conducted for identification and characterization of *Pasteurella multocida* isolates from various animals and avian species, by PM-PCR, HSB-PCR, multiplex-PCR, RAPD-PCR and REP-PCR. Outer membrane protein from *Pasteurella multocida* B:2 serotype, isolated and characterized by SDS-PAGE and Western blotting, revealed 2 major proteins of 32 kDa and 38 kDa, which were immunogenic in nature.

# **Bluetongue (BT)**

BTV vaccine using BEI inactivated bluetongue virus serotype 1 adjuvanted with saponin yielded promising results in experimental sheep. Inactivated vaccines against BTV type 1 and 23 were prepared. Field trial conducted with type 1 vaccine produced good



humoral immune response. BTV serotype 23 (Bhopal isolate) and BTV serotype 2 were passaged in cell culture for attenuation. BTV-23 got attenuated while some more passages of type 2 were required for attenuation of the virus. The 56.43% serum samples of sheep, goat and cattle from Punjab, Himachal Pradesh, Rajasthan, Uttar Pradesh, Uttaranchal, Maharashtra and Gujarat were found positive for BT antibodies by cELISA and AGPT. One of the 164 blood samples from Gujarat and AP was positive for BTV in RT-PCR. Type specific primer for typing of new isolates of BTV was designed. No appreciable variation was observed in nucleotide sequences of Indian isolates and Australian isolate of BTV 23. Per cent homology of nucleotide sequence of M5 gene of BTV Indian serotypes was from 38.6 to 39.1 with AHSV1 and AHSV 9 and 53.4 with EHD. Indian BTV types are closely related to each other forming monophyletic group. Full length S7 gene of the isolates is 1,154 bp along with a single ORF of 1,050 bp. Typing of virus isolates was done at the typing centre and the virus isolates of type 1, 2, 15, 18 and 23 were submitted to the repository. Samples of midges were identified as *Culicoides oxystoma*. A model trapper/ attractor was developed to capture midges.

# Animal health research in various livestock and poultry

**Equine:** A neutralizing monoclonal antibodies-based diagnostic kit Equi-herpes B-ELISA kit was developed. This kit tests the serum sample using single dilution (1:250) thus making it very economical. DNA fingerprinting of different EHV-1 strains revealed that more than one genetically variant strains of EHV-1 are circulating in equine population of Northern India. A patent application entitled "Neutralizing monoclonal antibody-based blocking ELISA diagnostic kit for detection of equine herpes virus-1 specific antibodies" is being submitted for getting Indian patent

# Quality control and production of veterinary biologicals

- A total of 270,000 doses of RD 'F' strain, 31,650 doses of lapinized swine fever vaccine, 149,310 ml of BPL-inactivated anti-rabies vaccine, 100,000 doses of tuberculin PPD, 10,000 doses of mallein PPD, 37,000 ml of Brucella agglutination test antigen, 3,960 ml of Brucella abortus Bang Ring antigen, 760 ml of Rose Bengal Plate Test antigen, 110 ml of Brucella positive serum, 2,000 ml of Salmonella Abortus equi 'H' antigen, 50 ml of Salmonella poly 'O' sera, 100 ml of S. Pullorum positive sera and 6,100 ml of S. Pullorum coloured antigen were produced and quality tested; 84,300 doses of tissue culture sheep-pox vaccine were tested for infectivity titre.
- Using FMD vaccine production technology in BHK-21 cell culture, 6.40 million doses of monovalent FMD vaccine were produced and 3.90 million doses of trivalent FMD vaccine were supplied.

for this diagnostic kit.

Extracts from medicinal herb *Lawsonia inermis* were evaluated to develop a dry treatment of *Trypanosome evansi* infection of equines. The results indicated that the constituents of *L. inermis* responsible for trypanocidal activity *in vitro* might have been degraded *in vivo* system when administered by oral or intraperitoneal route. For *in vivo* activity, a suitable drug delivery system needs to be evaluated to protect the allelochemicals.

A sandwich-ELISA was standardized for rapid diagnosis of rotavirus-associated diarhoea. This ELISA was 100% sensitive and highly specific as compared to virus isolation and RNA-PAGE.

Equine piroplasmosis caused by *Babesia equi* is a serious problem of equines in India. To develop improved diagnostics for this ailment, a truncated gene segment of one of the merozoite surface proteins, EMA-2 of *B. equi* was expressed in *E. coli* and the expressed soluble GST fusion protein was purified. An ELISA was standardized using this recombinant protein as antigen. The assay quantitatively differentiated the reference positive and negative serum samples. The assay was specific in detecting *B. equi* antibodies only.

Molecular diagnostics were developed for EHV-1 and EHV-4 viruses, *Streptococcus equi* subspecies *equi* and *S. equi* subspecies *zooepidemicus* and trypanosomosis. Sandwich-ELISA was developed for diagnosis of equine rotavirus infection.

Active sero-surveillance was conducted in Maharashtra, Rajasthan, Chandigarh, Delhi, Gujarat, Haryana, Himachal Pradesh, Punjab, Tamil Nadu, Chhattisgarh, Uttar Pradesh and West Bengal. EHV-1 antibodies were detected in 2.05% samples, while *Babesia equi* sero-prevalence was detected in 21.80% serum samples tested. Samples tested for equine infectious anaemia, African horse sickness, glanders, brucellosis and *Salmonella* Abortus equi were not found positive.

**Cattle:** The animals of the institute were screened for brucellosis using Rose Bengal Plate Test (RBPT) antigen and found that all samples were negative for the infection. The test was also compared with the positive serum.

*Mithun:* Abortus Bang Ring Test (ABRT) was used to detect the presence of antibodies against *Brucella abortus* in mithun milk. During the investigation, 30.8% of the total screened animals were found positive for *Brucella abortus*. These positive cases were confirmed further through A-B ELISA test. The result indicated that ABRT is a reliable test for detecting brucellosis in mithun. The immune response of mithun vaccinated with commonly available cattle FMD vaccine was evaluated. Approximately 80% of the vaccinated mithuns had protective antibody levels against serotypes '0'.

**Yak:** An overall prevalence of trematode, cestode, nematode and *Eimeria* species recorded in yaks were 6.74, 9.03, 76.22 and



35.07%, respectively, at Nyukmadung Farm. Prevalence of Neoascariasis in newly born calves has been identified as an emerging problem. *Parafilaria bovicola* and *Babesia bovis* in yak and its crossbreds were recorded for the first time. The ailing animals responded to the treatment.

**Sheep:** The data on health status of animals in breeding experiment showed that at Avikanagar, the breed-wise EADR was minimum in Malpura sheep (0.236) followed by Chokla (0.363), Bharat Merino (0.407), Garole  $\times$  Malpura (0.532), Avikalin crosses (0.628), Avikalin (0.698) and Garole (1.722). The agewise analysis revealed that EADR is inversely proportional to the age of animal. The major causes of mortality in adult flock were suppurative pneumonia, debility and JD. Among hogget and weaner major diseases encountered were toxaemia, suppurative pneumonia and urolothiasis. In sheep overall sero-prevalence of BT disease in farm flocks was 22.93%.

Period for higher prevalence of *Haemonchus contortus* and *Trichostrongylus* spp in population can be broadly predicted by the bio-climatograph. Studies on improvement of resistance to *Haemonchus contortus* showed that the susceptible progenies of Malpura breed had 3–5 times higher FEC (faecal egg count) compared to resistant progenies. Similarly in Avikalin breed the susceptible progenies had 2-4 times higher FEC values compared to resistant progenies.

At the ARC Bikaner, equivalent average morbidity rate (EAMR) was 0.1607 in Marwari and 0.20038 in Magra breed. The overall EAMR was 0.1559 (612 cases) per 1,000 animal days. The major clinical manifestations were pyrexia with haemorrhagic gastroenteritis (PPR) which occurred as an outbreak. The other manifestations were diarrhoea (16.3%), pneumonia (9.64%) and cases of general systemic state (8.49%). There were 12.90% cases of Johne's disease in Magra breed.

**Camel:** Trypanosoma evansi isolated from camel were maintained in liquid nitrogen at -196 °C under 12% (v/v) glycerol as cryoprotectant, for genetic homogeneity. On agarose gel (1.5%) electrophoresis a product range on first amplification revealed at 562 bp while on nested PCR using ITS-1 and intervening 5.8s, it comes around 500 bp. The sensitivity of all the reaction down to 1 to 10 parasites.

Faecal samples of farm herd camels were screened for different helminth parasites. The RAPD assay showed 6 distinct RAPD marker patterns ranging in size from 650 to 2000 base pairs with 2 different polymorphic patterns. Recovery of bacteria from more than 90% of the clinically infected quarters showed that in camel mastitis is mainly due to bacterial pathogens. Clots and flakes were found in the milk of the clinically infected quarters. Sodium concentration showed high increase in clinically infected camels but K concentration was almost same between milk of normal



Scanning electron micrograph showing PLG microspheres entrapped with combined RD&IBD virus (3000x)

and clinically infected quarters.

**Rabbits:** In rabbits at Avikanagar, the age-wise EADR ranged from 0.609 (adult) to 9.106 (weaner). Out of total necropsy, 73.6% belongs to weaners. Male rabbit had higher EADR (3.833) compared to female (2.966). Monthly EADR ranged from 0.148 (September) to 9.975 (March).

**Exotic diseases:** The sequence encoding E2 gene of bovine viral diarrhoea virus has been expressed in *E. coli* as truncated recombinant protein using pTriEx expression system. The matrix gene of Indian isolate of avian influenza virus (H9N2) has been sequenced.

#### Surgical and clinical interventions

Polyglycolic acid proved to be a better suture material than silk. Cyanoacrylate adhesive produced stronger bonding between urethral incised surfaces than fibrin glue. The role of ultrasound therapy was established in the treatment of hindquarter weakness, abdominal wall defects, arthritis and delayed fracture healing in rabbits and canines as one of the substitutes to conventional therapy. Acupuncture therapy was useful in the treatment of hindquarter weakness and posterior paresis in canines and caprines. The modified technique of interlocking nailing was successful in fixation of fractures in dogs with osteopenic bones. Attachable magnetic field stimulator was designed, assembled and tested for electromagnetic characteristics. Modified DS formulation with additional 'K' constituent was effective in clinical cases of diarrhoea in adult sheep and goats. Parental fluid containing sodium acetate as the alkalizing agent was the most effective low cost treatment in severe acidosis associated with calf diarrhoea. Parental fluid with sodium L-lactate was equally effective albeit higher cost. Nimesulide was found to be a more potent uterine relaxant in vitro indicating its potential use in abortion in goats.



# Development and improvement of diagnostics and vaccines

# **Diagnostics**

Dot-ELISA was developed for quick diagnosis of PPR infection in small ruminants. A rapid, highly specific and antigen capture RT/PCR was standardized and developed for the detection of PPR using the cell culture adopted PPR virus. Nested RT-PCR technique was quite effective for diagnosis of classical swine fever in field/ tissue samples. Nested RT-PCR primers targeted to highly conserved UTR were used efficiently for detection of infectious bronchitis virus isolates. ELISA was found quite sensitive for diagnosis of paratuberculosis in sheep and goats. Duplex PCR was found promising for confirmation of Mycobacterium a. paratuberculosis isolates in bovines. PCR assay was used to detect and differentiate the *Mycoplasma mycoides* subsp. *mycoides* type SC (MmmSC), the causative agent of CBPP, from the MmmLC and Mmc organisms. ELISA using purified F2, H2, cysteine proteases and/or metacercarial antigens was found useful to detect fasciolosis under field conditions in cattle and buffaloes. Good correlation between IFAT, dot-ELISA and PCR-assays in diagnosis of Babesia bigemina infection in bovine calves was observed. Duplex-PCR was found reliable for detection of Aeromonas from foods with rapidity, sensitivity and specificity. The PCR of 29 kDa gene was found useful for the molecular diagnosis of avian-pox virus infection in scabs and lymphocytes of the infected birds.

# **Vaccines**

Live attenuated goat-pox vaccine conferred complete protection up to 1 year post-immunization in goats, and the vaccine was safe in pregnant animals at the recommended field dose  $(10^2 \text{ TCID}_{50})$ . Foot-and-mouth disease cell culture-based vaccine using a metabolisable lipid emulsion adjuvant, elicited good antibody

# **Herbal medicines**

- In vitro trials on aqueous extracts of Jamun bark revealed reduction in goat-pox virus concentration by 4 log units.
- Alcoholic extract of Tinospora cordifolia (Golie), Curcuma longa (Haldi), Withiania somnifera (Ashwagandha) or juice of Allium sativum (garlic) either alone or in combination reduced the lead burden from the blood, liver and kidney, and lead-induced lipid peroxidation in the liver and kidney when tested in rats.
- Tinospora cordifolia and Withania somnifera (Ashwagandha) reduced the cadmium burden from liver and kidney in rats.
- Methanolic extract of Curcuma longa (Haldi) and aqueous extract of Emblica officinalis (Amla) exhibited 63% and 56% antibacterial activity, respectively, against bovine mastitis caused by Staphylococcus aureus, Streptococcus agalactiae and coliform bacilli.

## Development of an area specific mineral mixture for the livestock of Uttaranchal and Uttar Pradesh

Deficiency of essential minerals in soil vis-à-vis feeds and fodder adversely affects the health and productivity of livestock which thrive on these feeds and fodder. Consequently, the farmers face recurring economic losses, despite availability of adequate but unbalanced feed. Extensive survey in Uttar Pradesh and Uttaranchal, on the status of various essential dietary minerals in the soil, feeds and fodder and livestock (blood/ serum) revealed that the feeds and fodder grown on these soils were mainly deficient in phosphorus, zinc, copper and iodine followed by calcium, magnesium, cobalt and selenium of variable intensity. Most of the cattle and buffaloes of these 2 states suffered from composite mineral deficiencies evidenced by low production, reproductive and other clinical disorders, low blood/serum levels of several essential dietary minerals, etc. Supplementation of mineral mixture, formulated for a specific area, in diets of growing and lacting crossbred cattle and buffaloes for 90 days, significantly increased the growth rate and daily average milk yield, as compared to nonsupplemented group. The area-specific mineral mixture formulations are being successfully commercialized.



Lactating cow suffering from hypocalcemia showing lateral kinking of neck, characteristic of milk fever

response in vaccinated cattle under experimental conditions. Naked DNA-based vaccines developed against rabies, canine parvovirus, infectious bursal disease (IBD) and inclusion body hepatitis (IBH/ FAV4) were evaluated for their potential to generate protective immunity in experimental animals. Encouraging results were obtained in preliminary trials. Rabies glycoprotein gene and canine parvovirus VP2 gene were expressed in bicistronic expression (pIQEX) system. The combined vaccine was safe and potent when tested in dogs. Infectious bursal disease VP2 gene and exon gene of FAV4 (inclusion body hepatitis) based DNA vaccines gave encouraging results when used alone and/or in combination in chickens. Turkey-pox virus passaged in chicken embryos (25 passages) and CEF cell culture (40 passages) showed good protective index in chicks against challenge with virulent virus at second and eighth week post-



### Detoxified Karanj cake as protein substitute for economic mutton production

Karanj-cake, left as residue after oil extraction is hitherto wasted. Its 45 lakh trees have annual production

potential of 13 lakh MT of seeds from various parts of India, that could be utilized as a protein source for mutton production. Its use as animal feed is restricted due to the presence of toxic karanjin, a furanoflavonoid.

The growth rate, feed conversion efficiency, digestibility and retention of nutrients in lambs fed on diet containing water washed SKC (replacing 50% soybean-meal) in the concentrate mixture were comparable with that of control having soybean-meal as

protein supplement. Significant clinical manifestations were not

inoculation, respectively. The PLG microsphere based Ranikhet disease (F) vaccine provided good humoral and cell- mediated immunity including mucosal IgA antibody response and afforded protection against virus challenge up to 70% till sixth week postimmunization in poultry. The PLG microsphere based combined (Ranikhet disease + infectious bursal disease) vaccine afforded 60% protection against RD as well as, moderate resistance against IBD till sixth week post-immunization. The glycoprotein antigens of 34 and 29 kDa isolated from Hyalomma anatolicum anatolicum and *Boophilus microplus*, respectively, conferred protection against experimental challenge infections for at least up to 30 weeks. A saponified bivalent Pasturella multocida vaccine containing serotype B: 2 and A: 1 conferred 100% protection in buffalo calves against challenge for both the serotypes. A low volume (2 ml) saponified haemorrhagic septicaemia vaccine conferred up to 100% protection in calves challenged up to 12 months post immunization.

# Assessment of indigenous technologies

On-farm trial of indigenous technologies revealed promising efficacy of—pigeon waste to induce estrus in post-partum anoestrous animals; pigeon waste with jaggery to induce estrus symptoms in heifers; stone fruit (*Bel*), *takala* (*Cassia tora*) flower juice and *sheesham* leaves paste, juice of *urhul* (*Hibiscus sinensis*) flowers and paste of bark of pojo (*Litsaea anthapoly*) to check diarrhoea in small and large animals; *babool, jamun* bark and peach leaves with fresh milk in curing FMD lesions in animals; and *kala jeera* in haemorrhgic septicaemia.

# Animal feed resources and nutrition

The district wise information on feed resources for the last 15



observed in terms of blood biochemical and enzyme profile as well

as, immune response and rumen fermentation pattern in lambs fed diet containing water washed SKC. The dressing percentage and yield of primal cuts were comparable among lambs fed control and water washed SKC diets. No untoward taste or odour was imparted to the meat due to SKC feeding. Neither pathological lesions in different vital organs nor bone abnormalities could be noticed due to feeding of water washed SKC. The feed cost of unit meat production and live weight agin (kg) was reduced by Rs 19.83 and

6.83%, respectively, because of feeding of water washed SKC.

years was compiled and thematic maps were prepared. The information was brought out in the form of compact disc for Punjab, Haryana, Gujarat and Orissa. The compact disc is useful for developmental agencies and private industries to utilize the information for livestock development activities and planning business strategies.

Primary data on feeding practices, type of feeds/fodder/top feeds available, quantity offered, type of animals and socioeconomic aspects were collected from different categories of farmers in rainfed and coastal zones of Karnataka. Commonly available feeds and fodders and unconventional feeds collected were analyzed for micro- and macro-nutrients to identify the limiting nutrients on existing feeding systems. Intervention through strategic supplementation of deficient nutrients could improve productivity and profitability.

- District-wise information on feed resources compiled
- Paddy crop residues contain more moisture at harvesting compared to course grain crops
- Technology for detoxification of castor-cake passed on for commercialization
- Total mixed ration with 2 kg grain fodder proved economical ration for milch cow
- Encapsulated choline-chloride feeding increased milk yield
- Bromo-chloromethane capsule in diet of rams reduced methane production
- Fallen tree leaves @ 20% could be safely added to complete feed block
- Cu and Zn in diet improved micronutrients and mineraldependent enzymes
- Drying (80°C for 4-7 hr) reduced aflatoxin in diets
- Maize substitution up to 75% by sorghum proved economical feed for CARI-Sonali



The grain-straw ratios for major crops in Tamil Nadu and Andhra Pradesh from crop cutting survey data were worked out. The grain-straw ratios for paddy in Andhra Pradesh and Tamil Nadu were 1 : 1.14 and 1 : 1.06, respectively. In Tamil Nadu, the pod-haulm ratio for groundnut and cane-top ratio for sugarcane was worked out to be 1 : 3.5 and 1 : 0.21, respectively. The analysis of driage co-efficients of crop residues revealed that paddy crop residues at the time of harvest contain more moisture (50 to 58%) compared to coarse grain crops like *jowar* (48%) and groundnut (45%). Estimation of driage coefficients of major crops for other states of the country is in progress.

# Cattle

Utilization and treatment of conventional and nonconventional livestock feed: Technologies were developed for detoxification of castor-cake and utilization of sunflower heads as livestock feed. The detoxification technology for castor-cake was passed on for commercialization. The technologies have resulted in addition of 6 lakh tonnes of high protein castor-cake and 11 lakh tonnes of sunflower heads as potential feed resources. Roasted soybean-cake (160°C for 30 min) proved to be a good source of by-pass protein in the diet of ruminants. A methodology was optimized for determination of 18 organophosphorus and 10 more persistent organochlorine pesticides using HPLC. A binary gradient elution was developed which separated all the components within 60 min run. Formaldehyde treated mustard-cake could be fed with its improved bypass protein value, and it enhances the production performance of the lactating crossbred cows. Babul pods could be used as a source of energy in ruminant ration, and could be incorporated up to 20% in the concentrate mixture of lactating crossbred cows. Use of 2% activated charcoal (AC) as an antidote given to growing Karan Fries (KF) male calves, being fed on a diet containing 25 ppm chlorpyriphos (CPP), showed a protective effect in the diet of crossbred calves. Monensin supplementation significantly increased growth rate in crossbred heifers. Feeding of a total mixed ration containing concentrate: wheat straw in the ratio of 70: 30 with 2 kg green fodder resulted in production of milk at a cheaper cost from crossbred cows compared to the diet containing higher quantity of green fodder. Encapsulated choline chloride (ECC) feeding increased milk yield by 7.3% (0.911 kg/animal/day) and increased milk fat content by 4.8%, and lactose by 3.9%. The As and Cd content in feed samples collected from some industrial towns in Haryana were higher than the maximum permissible level. The As is released up to 97% following its dietary supplementation at 50 ppm. Dietary Cd supplementation @ 10 ppm reduced Zn and Cu retention in crossbred cows. Analysis of heavy metals in soil, fodder, feed and milk indicated that lead and cadmium were at non-detectable

#### Improving locally available feed resources

*In vitro* and *in* sacco degradability studies of different green forage and concentrate based total mixed ration have been conducted. The *in vitro* and *in* sacco degradability of dry matter and organic matter were similar. However, *in* sacco degradability of crude protein was considerably higher compared to *in vitro* degradability. The study indicated that probably the coarse fodder utilization capability is considerably higher in mithun.

levels in all samples from all areas analysed. The Cu, Mn and Fe were high in almost all samples, whereas Zn was deficient in most of the samples. Supplementation of zinc in the diet improved the cell-mediated immune response in buffaloes. Crossbred cattle (112) having reproductive problems were screened for their micronutrient status. The problematic animals were supplemented with deficient area specific mineral salts. All the supplemented animals showed improvement in health condition within 15–30 days of supplementation. Anoestrus animals of sexual maturity cycled within 30 days, animals below 2 years age cycled in 3–4 months. In all about 80% of cattle supplemented with mineral salts got confirmed pregnancy. Supplementation of area-specific mineral mixture increased growth rate and daily average milk yield.

**Detoxification and nutritional improvement of unconventional and poor quality feeds:** Hydrolysable tannins, condensed tannins and saponins at 5% (w/w), significantly reduced total gas production *in vitro* in wheat straw, white clover, subabool (*Leucaena leucocephala*) and mulberry (*Morus alba*). Condensed tannins at 5% level gave significant protection against protein degradation, and saponin (1.3 mg/ml) revealed maximum microbial protein synthesis and efficiency. Bacterial biotransformation of the toxin precocene-II resulted in a gradual decrease in its concentration and complete utilization of the toxin at 18 hr of incubation. Supplementing bromo-chloromethane capsule in diet reduced methane production in rams without any adverse effect on their performance. *In vitro* studies with different bioactive compounds and inclusion of urea in the diet of ruminants also decreased methanogenesis in the rumen.

**Biotechnological approaches for increasing productivity:** Isolation and characterization of *Butyrivibrio fibrisolvens* was carried out and different strains of the organism were obtained. New shuttle vector pBS43 was used for cloning the cellulase gene and transformed *Escherichia coli* DH5alpha. Transformation with *Butyrivibrio* revealed that the organism was refractive to transformation.

Solid state fermentation (SSF) of green hybrid napier (steamed ST), untreated (UT) and urea treated (UT) was carried out using





Rumen fungal species isolated from blue bulls and goats

Goats have tough time in digesting the pasture, as grasses dry off during summer, peak winter and drought. Under these unfavorable conditions, goats may hardly digest 40–50% of what they eat, which affects their productivity. Some exotic animals like nilgai (Boselaphus tragocamelus) in the same habitat sustain their productivity under such a harsh condition, owing to presence of improved strains of anaerobic fungi in their rumen. Potential role of anaerobic fungi is exhibited by the considerable evidences which show positive relationship between presence of anaerobic fungi in the rumen and voluntary intake of herbage diets of low digestibility. Association of rumen fungi with improved digestibility of poor quality roughages containing higher proportion of lignin and fibre is also established. Neocallimastix, Piromyces and Orpinomyces were found in rumen of both nilgai and goats. Improved activities of carbomethyl cellulase, microcrystaline cellulase, xylanase and B-glucosidase in the rumen of nilgai were observed. Amplification of cellulase C gene (430 bp approximately) using PCR against specific primer confirmed the presence of Orpinomces isolated from the rumen of blue bulls (nilgai) at molecular level. Information on fibre digesting enzymatic activities of rumen fungi from both host species are being collected for inoculation of efficient strain of exotic rumen fungi in goats rumen for improved intake, digestibility and productivity on low grade roughage.

*P.sajorcaju* to assess the changes in its proximate composition and enzyme profile. Protein content of hybrid napier increased in the UT and ST after fermentation. Lignin content decreased in the fermented samples. Properties of CM cellulase isolated from *P. sajorcaju* were elucidated.

# **Buffalo**

At the CIRB Sub-Campus, Nabha, nutrient requirements of Nili Ravi heifers were estimated at 103.3 to 129.5 Kcal/kg  $W^{0.75}$  ME at various body weights and the maintenance requirement for crude protein was found in the range of 6.49 to 9.49 g/kg  $W^{0.75}$ .



For 1g gain in body weight, the growth requirement of metabolisable energy was in the range of 6.18–12.91 Kcal at different body weights, while for CP these were 0.24–0.42 g and for metabolisable protein these were 0.18–0.31 g. Nutritional requirement of buffaloes during the last quarter of their pregnancy could be fulfilled by feeding them on a ration containing medium protein (10.5% DCP) and high energy (58% TDN). Buffaloes fed on such ration gave birth to healthy calves and yielded more milk. Buffaloes fed on TMR showed higher milk production than on conventional feeding system on a peri-urban dairy.

**Feeding practices:** Survey of feeding practices in two districts of Haryana State indicated that the feeds and fodders fed to buffaloes were deficient mainly in zinc, manganese and copper. This was responsible for a high incidence of anestrus in rural buffaloes, which could be corrected by feeding area specific mineral mixture formulated at the institute for particular areas. On comparison of daily intake requirements prescribed by feeding standards, animals kept by farmers in Punjab were receiving calcium, phosphorus, zinc and copper at 90, 48, 39 and 39% levels, respectively, which accounted for moderate to severe deficiency of these minerals.

**Complete feed blocks for buffaloes**: Keeping in view the constraints of storing dry fodder and importance of making available balanced ration to buffaloes of various categories, 'complete feed blocks' were prepared. Feeding of these blocks improved the overall dry-matter intake and further improved digestibility of crude protein, ether extract and fibre.

*Cellulose and xylanase mixture:*Supplementation of mixture of cellulase and xylanase improved the digesting and daily body weight gain from 510 to 560 g in buffalo calves. Feeding of green fodder enhanced the total CLA content in buffalo milk.

# Sheep

**Development of feed and fodder resources:** Dry fodder yield and biomass production of groundnut in strip cropping was significantly higher as compared to sole cropping. At the ARC Bikaner, the weed free field produced maximum fodder yield with maximum net profit.

**Performance of sheep flock maintained on degraded rangeland:** Performance of sheep flock maintained on degraded rangeland located near the institute, was monitored. A strategic supplementary feeding schedule based on the experiments conducted during the last 3 years on feed intake and nutrient utilization in sheep grazing on community grazing lands during different physiological stages and different seasons in semi-arid region of Rajasthan, was developed for adoption under the field.

*Evaluation of fermentation rate using* in-vitro *gas production technique:* Different concentrate ingredients commonly

### Improvement of feed resources and nutrient utilization in raising animal production

Information related to different livestock species and availability of feed resources, their nutrient contents as well as information on traditional feeding system in the north eastern region is being compiled. *Blemkar*, *domkar*, *sylulli* and *phrengpa* trees are commonly used as fodder in Arunachal Pradesh for feeding yak. In Sikkim, trees are commonly used fodder, *amilisho*, *nebaro*, *gagun* and *ber* for feeding of livestock.

used in sheep feeding were evaluated for fermentation rate kinetics using *in vitro* gas production technique. Sheep with poor growth rate when fed on 300 g/day concentrate supplement with trypsin, showed significant increase in the digestibility of fibre and decrease in fat digestibility. However, there was drastic decrease in microbial protein production (50%) and nitrogen balance (60%) in these sheep.

**Feeding of complete feed block during scarcity period:** During drought or famine, fallen tree leaves can be used as a component of complete feed block (CFB), and can safely be added up to 20% level. In the nutritional studies for mutton production it was observed that the dry matter intake in preweaning phase was higher in lambs fed on butyrate dose. Chokla rams receiving (25%-above-requirement) ration produced more greasy fleece (1,058 g) than, rams fed on below required or normal ration.

**Bioavailability of micronutrients:** Bioavailability of Cu and Zn in sheep was 43.20 and 24.80% more when supplemented through organic sources (chelated–AA) compared to inorganic sources. Availability of Ca and P were better in adult sheep fed organic sources of Cu and Zn even at higher levels of these minerals in the diet. Digestibility of DM and OM did not differ significantly in sheep fed diets supplemented with organic and inorganic sources of Cu and Zn. Micronutrients (Ca, Mg, P, Cu and Zn) status and mineral-dependent enzymes (ceruloplasmin, Cu-Zn superoxide dismutase, alkaline phosphatase) activities in blood plasma were studied in sheep fed different levels of Cu and Zn in the diets. Increased supplementation of Cu and Zn in the diet resulted in increased levels of these minerals and mineral-dependent enzyme activities in the blood plasma.

*Feeding of afltoxin contaminated feed to sheep:* Drying of aflatoxin contaminated feed in hot air oven (80°C for 4–6 hr) or in natural sunlight (2 days, 14 hr) resulted in significant reduction in aflatoxin content. Feeding of aflatoxin contaminated feed to sheep after drying either in hot air oven or in sunlight did not affect the performance. Hence, drying of feed is quite effective and practical way of reducing the aflatoxin content.

#### Goat

**Organophosphorus pesticides in animal feed and their excretion in goat:** A binary gradient elution programme on HPLC was developed for the determination of 18 commonly used organophosphorus pesticides under Indian conditions and 10 persistent organochlorine pesticides. About 85% monocrotophos was degraded within 48 hr of incubation. Under *in vitro* conditions activated charcoal was a better antidote than zeolite. On supplementing activated charcoal to a diet contaminated with monocrotophos in lactating goats, nutrient digestibility and acetocholinesterase, serum creatinine and serum transminases (GOT and GPT) increased. There was 27% reduction in the excretion of monocrootphos in milk.

#### Rabbit

At the NTRS Garsa, Himachal Pradesh, adult Angora male fed on 110 g of concentrate/day required 7.84 kg dry matter for producing 100 g of wool, and rabbits fed on 80 g concentrated and 250 g fresh mulberry leaves required 9.12 kg dry matter for producing 100 g wool.

# Pig

At the AICRP on Pigs, Tirupati centre, 100% fish-meal was successfully replaced in pig ration with amino acids supplement. The IVRI centre recommended the feasibility of lowering the use of maize in pig ration from 50 to 35%, and increasing the percentage of wheat bran to 47% from around 20%. Nutritional trial in the Khanapara campus of AAU, indicated the possibility of replacing the concentrate requirement up to 30% with colocasia, fish-meal with silkworm pupae and mustard oil-cake.

# Camel

Evaluation of locally available feed and fodder: The lactating camels given complete feed blocks (CFB) indicated significantly higher average daily gain (1,117.42 g/d) than the MC group (227.27 g/d) indicating that feeding management system has profound effect on body weight changes. While feed refusal was 27.66% of offered fodder in MC, it was 10.58% in CFB indicating that if all the feed ingredients are mixed and compressed into feed blocks, the wastage of feed will be significantly reduced. Further feed intake also significantly increased in CBF group increasing milk yield and composition. The DMI (kg/100 kg body weight) was higher in CFB. Higher CP digestibility in complete feed blocks was attributed to higher dietary CP level. Complete feed blocks promoted rumen microbial growth and activity thereby resulting in increased digestibility of poor quality roughages. The feeding of CFB to camels resulted in significantly higher intake of DM, DCP and TDN on metabolic body weight basis. Higher



intakes of DCP, TDN and ME values in CFB fed camels were on account of higher DMI and digestibility.

The average intake of Na, K, P and Mg was higher in CFB.The drinking water was significant source of Na macro-mineral as compared to other minerals and accounted for 22-45% of total Na intake/d. Significantly higher apparent absorption of Na, Ca, P and Mg from the gut was recorded in CFB camels as compared to those of MC camels while K absorption was statistically similar between 2 groups because the potassium levels are generally higher in all plants and their parts.

#### Yak

Information regarding common husbandry practices and feeding regimes, viz. feeds, fodder and tree leaves commonly fed to the yaks, was collected from some of the yak rearing pockets of Arunachal Pradesh. Proximate composition of feeds, fodders and tree leaves was estimated.

# Mithun

The nutrient digestibility and level of tree leaves incorporation were estimated in total mixed ration (TMR) for mithuns. Addition of mixed tree leaves up to 50% of TMR was good green roughage source to meet the nutrient requirement in mithun.

# Poultry

**Updating nutrient requirements and their bioavailability:** Requirements of laying quails, heavy body weight and white breasted lines, developed at the CARI were—protein 18.2%, lysine 1.0%, methionine 0.45%, threonine 0.74% and metabolizable energy 2,900 kcal/kg of diet; and the dietary protein could be reduced by 1.8% in diet provided the ideal amino acids profile is maintained. The CARI-Red chickens developed at the CARI require metabolizable energy (ME) 2,800 kcal/kg, CP 11.9% and lysine 0.47% during growing stage (12–21 weeks); ME 2,600 kcal/kg, CP 16.2%, lysine 0.80% and methionine 0.35% from 24– 36 weeks laying period; and ME 2,600 kcal/kg, CP 14.7%, lysine 0.6% and methionine 0.30% during 36–48 weeks of laying. The naked neck × WL layers required a dietary level of 2.5% calcium and 55 mg/kg zinc for optimum utilization of different minerals.

*New feed resources for poultry feeding:* Diets containing different proportions of pearl millet, either as such or reconstituted without or with enzyme addition accrued similar body weight gain, feed intake, and feed conversion efficiency in growing quails. Meal and sunflower seed-meal in combination either in maize, maize–pearl millet, maize–sorghum and pearl millet–sorghum based diet replacing soybean-meal supported optimum egg production during second phase of laying cycle of CARI-Sonali hens. Diet containing sunflower-meal as sole protein



supplement with enzyme accrued more egg production with superior feed utilization efficiency. Partial replacement of maize up to 75% by sorghum or utilization of cereal mixture (maize + sorghum + pearl millet @ 1:1:1) was beneficial for economic production of CARI-Sonali hens.

**Augmenting nutritive value of poultry feeds:** Probiotics, viz. MOS or FOS (1 g/kg) and lactose (30 g/kg) could be used as suitable substitute for growth promoting antibiotic BMD) in broiler quail. Optimum dietary levels of probiotics in laying quails feed were 1 g/kg for MOS and FOS and 20 g/kg for lactose, which had a positive influence on production performance, immune function and competitive exclusion. Formic acid (1%) and propionic acid (1%) in diet, significantly increased body weight gain, feed efficiency and reduced microbial count in feed and caecal contents. Inclusion of 0.4% aluminium as aluminium sulphate in normal layer diet for 8 days was beneficial as assessed by post-moulting performance of hens.

**Incriminating substances in diet and their amelioration remedies:** Broiler chicks could tolerate 30 and 15ppm of fenvalerate and methyl parathione, respectively, in diets containing enhanced mineral supplements. Interaction of castor bean-meal (CBM) toxin with fenvalerate and methyl parathion was significant and synergistic in broilers. The detoxification methods used for CBM (roasting + KI, 35 ppm) and insecticides (activated charcoal, 0.25%) appreciably reduced the concomitant toxicity resulting out of the interaction.

# Cattle

*Effect of physiological and nutritional intervention:* Deficiency of certain minerals, especially zinc and high blood urea nitrogen levels were possible causative factors in several animals with reproductive problems. Nutritional supplementations/ modulations are being made to ameliorate the problems and follow up is being done.

# Buffalo

Heat exposure reduced the lymphocyte proliferation rate and increased plasma cortisol levels in growing and adult buffaloes. Heat load was higher in Karan Fries cows compared to Sahiwal and buffaloes. Different mitogens affected lymphocyte proliferation index in growing buffaloes. Response of phytohaemagglutinin (PHA), tetanus toxoid and Con A was more in 1–2 months old animals compared to 3–4 months old or adult buffaloes. Photobiotin and photodigoxigenin bleached DNA expression and cloning vectors could be efficiently used for transfection of capacitated and electroporated buffalo spermatozoa to act as a vehicle for the transfer of the same to the ova. The analysis of expression and

- Isolation and purification of buffalo pituitary gonadoptropin hormone are being carried out
- Addition of glycerol at initial stage of dilution increased frozen semen production
- Ram effect was more pronounced in multiparous ewes
- Sirohi bucks appear to be the best suited to combat thermal stress
- Rumen of a goat seems to act as water reservoir and might meet the animal's demand when grazing away from water source
- Effect of freeze-thawing on goat semen was studied
- For estrous synchronization in pigs, 300 and 340 mg progesterone was suitable
- Modulating prolactin levels improved egg production in non-descript birds also
- Growth promoters improved growth up to 2 weeks of age in poultry
- Egg production in quails stopped in stressed birds
- Stress increased fat deposition in abdomen, breast and liver muscles
- Estrus was successfully synchronized in yaks by using  $\text{PGF}_2\alpha$  and GnRH

localization of mRNA encoding IGF-I and IGF-II in buffalo ovary was done by semiquantitative RT-PCR. Both IGF-I and –II were expressed in whole follicles of different sizes and granulosa cells from these follicles. Amplified partial IGF-1 cDNA was cloned in PCR-II vector (*in vitrogen*) and got custom sequenced. TNF- $\alpha$ and its receptor-1 (TNFRI) were expressed in whole follicles, granulosa cells from the follicles and post-ovulatory structures in buffalo ovary. FSH(100 ng/ml) showed stimulatory effect on expression of IGF-II and TNF- $\alpha$  in granulosa cells *in vitro*.

Anestrus in peri-pubertal buffalo heifers and early postpartum buffaloes were studied with ultrasonographic monitoring of ovarian activity and progesterone profiles. Attempts were made to simulate the physiological events associated with development, growth and ovulation during spontaneous estrous cycle, with exogenous hormonal supplementation. Mimicking luteal phase with exogenous administration of a synthetic analogue of progesterone in anestrus buffaloes, supplemented with eCG, not only induced estrus in approximately 90% of treated animals and resulted in good fertility of over 60% to fixed time inseminations. Using Ovsynch-Plus it was found that ovulations in response to second GnRH were essential for conception in treated animals.

The high incidence of backward motility in buffalo semen frozen during summer, resulting in rejection of a large number of ejaculates, was overcome with a modified semen freezing protocol involving addition of glycerol at initial stage of dilution. This protocol resulted into successful freezing of approximately 20% more ejaculates, leading to increased frozen semen production. Stage of glycerolization did not affect morphology of buffalo



# Isolation and purification of buffalo hormones

Hormones and related biological materials are not easily available and are quite expensive. Such materials are especially not available for buffaloes. To overcome this, isolation and purification of buffalo pituitary gonadotropic hormones, production of antibodies to them as well as for steroid hormones are being carried out. Isolation and partial purification of FSH and prolactin were completed. FSH activity in the partially purified extract was studied *in in-vitro* culture. Antisera for progesterone and oestradiol were raised. The complete nucleotide sequences of coding exons of buffalo growth hormone gene were worked out and some unique substitutions of nucleotides were identified. Sequences of the beta-subunit of luteinizing hormone gene cDNA were deduced and at least five different SSCP variants were identified.

spermatozoa, except the finding of a high incidence of sharp kink in the tail, which appeared associated with the apparent backward motility of sperm cells. The spermatozoa from fertile buffalo bulls were better equipped to combat the oxidative stress compared to the infertile bulls in terms of antioxidant enzymes and antioxidant status. The level of production and mode of action of reactive oxygen species (ROS), viz. hydrogen peroxide and superoxide anion during *in vitro* capacitation of buffalo spermatozoa was established. Buffalo sperm *in vitro* capacitation by superoxide anion ( $O_2$ ), hydrogen peroxide ( $H_2O_2$ ) and nitric oxide (NO) was modulated through tyrosine phosphorylation of different groups of proteins. Nitric oxide modulated the tyrosine phosphorylation of p38 and p20 whereas  $O_2$  and  $H_2O_2$  modulated the phosphorylation of p95 and p78.

Various functional tests on semen-seminal plasma as well as sperm were standardized for buffalo semen. Preliminary work on identification of molecular markers revealed differences in protein electrophoretic profile in semen from bulls of different fertility status.

# Sheep

Active immunization against inhibin-based peptide immunogens induced multiple ovulations and increased the ovulation rate from 3- to 7-folds over nearly 3 years period in Malpura sheep. Collection of transferable quality embryos and the birth of live offspring from inhibin immunized Malpura sheep confirmed that the multiple ovulations resulted in production of viable embryos which could produce live offspring. 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 compared to nulliparous ewes. Progesterone levels estimated in blood samples collected during isolation period from January to March suggested that this breed of ewes enter into anoestrus season during February. The thyroid hormone profile revealed that the thyroid activity is reduced during non-breeding season. Further this approach can be used to advance the age of sexual maturity in sheep. The body condition scoring (BCS) of the 88 adult Bharat merino ewes was evaluated. The ewes having 2.5, 3.0, 3.5, 4.0 BCS were marked as group 1, group 2, group 3 and group 4, respectively. The intensity of sexual behaviour was lower in 2.5 BCS as compared to 3.5 BCS ewes. The estrus duration of group 1 ewes was significantly lower compared to group 2 ewes.

*Freezability of Garole sheep embryos:* Embryos (14) of morula stage were frozen using glycerol based freezing media and slow freezing method in programmable cell freezer. Active immunization during off-breeding season (winter) did not result in improvement of ovulation rate as compared to that of control after first and third booster dose. The forced RFLP-PCR technique confirmed the Garole lambs produced through embryo transfer. The study indicates possibility of producing Garole lambs of heavier birth weight in large size recipients for their faster multiplication and conservation.

*Single transcervical artificial insemination technique:* A fertility trail was conducted in 100 cycling Bharat Merino ewes by single transcervical artificial insemination in 1 cycle using frozen-thawed semen. The ewes that did not return to estrus after two cycles were subjected to ultrasonography between 40 to 60 days for confirmation of pregnancy. The number of ewes that were marked conceived on non-return to estrus and pregnancy diagnosis were 25 and 20, respectively.

# Goat

**Adaptability of goats:** Sirohi, Jamunapari, Marwari and Barbari goats were evaluated for their adaptability during summer. Sirohi and Jamunapari bucks were able to loose excess heat through more efficient mechanism of higher sweating rate. Marwari bucks used respiratory evaporative heat loss to a greater extent than other breeds mainly due to their long shaggy hair coat that interfered in cutaneous evaporative heat loss. Barbari bucks utilized both efficient sweating mechanism and higher respiratory evaporative heat loss. The higher demand of heat loss in Barbari bucks was possibly because of more heat load due to higher metabolic rate in this breed compared to other 3 breeds. The cardiac activity in Barbari was also higher than the other 3 breeds.

Sirohi bucks had consistently significantly lower mean rectal temperature in all the thermal environments than that in Marwari, Jamunapari and Barbari bucks indicating inherent lower body temperature of this breed. The metabolic rate was also lower in



this breed compared to the other 3 breeds. The bucks of all the 4 breeds reduced their metabolic rate during hot thermal environment. Water intake and urine excretion in Sirohi bucks was also significantly lower than the other 3 breeds. Physiologically, Sirohi bucks appear to be the best suited to combat the thermal stress in semi-arid climate.

Exposure to solar radiation during cool period resulted in greater reduction in metabolic rate in black Marwari and dark tan Sirohi goats when compared with corresponding metabolic rate under shade in cool period and under solar radiation during hot period. Absorption of proportionately more incident solar radiation impinging on animal body appears to be beneficial during cool period as this energy can be utilized for thermoregulatory needs of the body. Black or dark coat colour in breeds of desert i.e. Marwari and Sirohi seems to be thus an adaptation for energy economy. Thus, the critical season of the year for the desert goat like Marwari seems to be the winters. Considering the fact that winter is the time when food in the desert is least available and energy requirements of the goats for thermo-regulation and for reproduction are high, any saving of their energy expenditure may indeed be crucial for their survival.

During water scarcity, goats conserved body water excretion by decreasing feed intake and urine excretion, voiding faeces more drier and reducing sweating rate. Water deprived goats allowed their body temperature to rise with decreased respiratory rate. Under normal conditions when water is available *ad lib*, a rise in body temperature will be accompanied with increase in respiratory rate. In water deprived bucks, respiratory rate declined to reduce the water loss through respiratory evaporation. An increase in the heart rate due to water deprivation was observed in all the 4 breeds and in three environments. It is expected that water deprivation will reduce the metabolic heat production, since feed intake is reduced. Heart rate under such circumstances should show a declining trend, but reduction in plasma volume due to dehydration and increase in blood viscosity seems to be the probable reasons for increase in heart rate in the present investigation.

The bucks quickly replenished their body weight when drinking water became available. The amount ingested compensated approximately the same to that of the amount lost during dehydration in cool and moderate periods. In hot environment, the bucks imbibed more water than their body weight loss. The rumen of goats seems to act as water reservoir as it drinks voluminous amount of water in few minutes. This rumen water reservoir might meet the animal's demand while grazing far away from water sources in arid conditions.

*Cryopreservation of* in-vitro *produce embryos:* Cryopreservation of *in vitro* produced embryos at 2- to 8-cell stage were performed using 1.5M glycerol as a cryoprotectant by conventional freezing protocol.

Hormonal profile and ovulation characteristics in Jamunapari goats: Level of circulating progesterone hormone in Jamunapari ranged from  $0.58\pm0.12$  ng/ml to  $8.35\pm2.60$  ng/ml; concentration remained lowest ( $0.58\pm0.12$  ng/ml) on the day of oestrus and increased gradually to reach a peak level of  $8.35\pm2.60$  ng/ml on the day 14th of the cycle and again reached to basal level on the day of oestrus ( $0.40\pm0.15$ ng/ml).Ovulation in this breed occurred during 30-36 hr following natural oestrus. Right ovary was more functional than left. The optimum time for mating to maximize reproduction rate in this breed is 12 hr after the onset of oestrus.

**Enzymes and proteins in goat reproductive fluids:** Analysis of follicular fluid, cervical mucus, uterine fluid and seminal plasma revealed that maximum total protein concentration was in follicular fluid followed by seminal plasma while uterine flushing and cervical mucus contained much less total protein. The acid phosphatase, alkaline phosphatase and GOT activity was also highest in seminal plasma compared to enzyme activities in uterine flushings, follicular fluid and cervical mucus. GPT activity was higher in follicular fluid and seminal plasma, uterine flushing and cervical mucus possessed nearly similar enzyme activity.

# Equine

**Cryopreserved semen of Marwari horses:** Equine semen is far less tolerant to the process of cryopreservation as compared to other species. Concentration of glycerol and equilibration time affects the post-thaw motility of frozen semen. Freezing media containing 3% glycerol was superior than that containing 5% glycerol on the basis of post-thaw motility, whereas equilibration time had no effect on stallion semen freezability. Thawing at  $45^{\circ}$ C for 15 sec was superior for obtaining better post-thaw motility in frozen stallion spermatozoa.

# Camel

**Work performance of camel:** The camel can pull one tyne plough for longer duration (5 hr 33 to 43 min) than two (4 hr 40 to 44 min) and three tyne (4 hr 10 to 22 min) ploughs. But, three tyne plough cover more area (5,135 to 6,020 m<sup>2</sup>) in relatively shorter period of time as compare to two (5,341 to 5,782 m<sup>2</sup>) and one tyne (5,721 to 5,821 m<sup>2</sup>) ploughs. Camel farmers reported that one tyne plough is most commonly used than two and three tyne ploughs. The farmers were of the view that their camels can work throughout the day with one rest of around 2 hr with one tyne plough.

*Evaluation of camel milk:* Significant higher value of lysozyme activity was observed in camel colostrum in comparison



of camel milk. The pH of camel whole raw milk samples were adjusted to 6, 5, 4, 3 and 2 using 0.1 acetic acid. The highest loss of 95% in insulin activity was recorded at pH 2 and 3.

**Developing RIA facilities for reproductive hormones in camel:** Estradiol-17 beta (E2) and progesterone (P4) profiles were studied in camels at different reproductive stages.

**Peripheral plasma progesterone profile:** Peripheral plasma progesterone profiles were monitored from day 0 to day 29, either daily or on alternate days to evaluate efficiency of hCG to induced ovulation. Nearly 40% of buserelin treated and inseminated female camels have definitely ovulated and developed a normal corpus luteum.

# Poultry

*Effect of modulating prolactin blood levels on egg production:* Physiological approach of modulating blood prolactin hormone levels in birds showed an increase in egg production by about 4% in farm layer birds. Approaches of immunizing against prolactin or vasoactive intestinal peptide (VIP) and controlling prolactin release using bromocriptine were applied. Girirani birds showed an increased egg production of up to 15% (from 25th weeks of age to 70th week of age) in birds immunized against VIP and prolactin. Against a normal of 160–180 eggs for one production cycle, the maximum egg production went up to 240 eggs. This showed that modulating prolactin levels improve egg production even in non-descript birds.

*Effect of estrogen—progesterone on egg production:* Egg production in *desi* fowl (Assel × Kadaknath) increased up to 4 egg/bird by administrating the estrogen-progesterone preparation at 15th week of age for 15 days of supplement in feed. Egg production in *desi* fowl (CARI Red × Assel Pela – CARI Nirbhik) could be increased up to 6.00 egg/bird from 20–33 weeks of age by administrating the drug through feed for 9 days at 21st week of age. It was further concluded that the pre-pubertal age of bird is extremely critical to administer the drug for enhancement of egg production. This critical period vary from species to species.

**Standardized melatonin estimation by HPLC:** Melatonin level in intestine (jejunum) was estimated using high performance liquid chromatography (HPLC). The HPLC method was standardized by altering stationary and mobile phase, buffer etc. This method gave satisfactory results. The sensitivity was 20 pg and detection time about 8 min.

*Standardization of pinealectomy technique:* Pinealectomy was performed in 1-2 day-old chicks under general anesthesia. The technique for pinealectomy in day-old chicks was standardized but still more refinement is required.

**Rearing and management practices of turkey:** During 6–10 weeks period the turkey poults raised under cage brooding

# Rutting behaviour of camel under different management conditions

Rutting behavioural frequency and intensity parameters of daily exposed male group (close to adult females) and unexposed group did not indicate difference in behavioural pattern up to third week of November (onset of winter). From last week onward all these parameters became apparent, and significant differences were observed between 2 groups. The plasma cortisol and testosterone levels varied significantly between groups from fourth week onwards. The DM intake and body weights of groups differed significantly from third week onwards. Early sexual behavior/rut signs in adult mature male camels are apparent when exposed close to adult female camels (regularly for 20–30 min daily) at least for 2 weeks during onset of winter.

systems, weighed 240 g higher compared to floor brooding systems. At 10–12 weeks of age, floor reared poults had better FCR than that of cage reared poults (3.0 vs 3.47). Humoral immune response ( $\text{Log}_2$  value of HA titre in response to 1% SRBC) was better in floor reared poults (8.75) than those in cage rearing system (6.91). Body weight of poults fed either of the growth promoters (virginiamycin and prebiotics) up to 2 weeks of age improved significantly. Both the growth promoters increased body weight at 4 weeks of age but, virginiamycin supplemented feed further increased the body weight significantly up to 8 weeks of age. Thereafter, the growth promoters failed to increase the body weight. There was no difference in the FCR values even if the growth promoters were supplemented in the diet.

**Manipulation of embryonic and post-hatch growth:** Glucose injection on either 14th or 18th day of incubation in to the yolk sac of embryo had better chick to egg weight ratio, and higher levels of plasma protein and uric acid levels at day-old, implying higher anabolism as well as catabolism of protein. This site (yolk sac) can be used as route for future *in ovo* injection. Carbohydrate (glucose, fructose and ribose) injected birds obtained 33–42 g more weight at 4 weeks of age than that of control. Humoral immune response ( $Log_2$  value of HA titre in response to 1% SRBC) at 14–21 days of age was higher in glucose and fructose compared to un-injected control birds; however, cellmediated immune response (response to *in vivo* PHA-P) studied at 19th day did not show any significant difference between carbohydrate and control group.

**Environmental pollution in poultry housing**: Ammonia (10–30 ppm) and carbon dioxide (375–600 ppm) gases measured in the different turkey sheds were within the range of recommended level. The dust concentration ranged from 0–7.81 mg/m<sup>3</sup> in turkey houses during early summer.

Chronic stress effects: Average daily feed intake in group S 1



#### **Effect of stress in Japanese quail**

The brain AChE activity (membrane bound plus cytosolic) in chronic stressed group was significantly higher as compared to the control and acute stressed groups. The AChE activity increased as the stress prolonged. The serum AChE also followed the similar pattern. Egg production was stopped completely in both the chronic stress groups after 6 days of stress. The birds of the fourth group started laying by the end of the ninth day of stress. Egg weight, albumen weight and yolk weight except body weight did not show any significant difference between treatment and control groups. The end products of nitric oxide (nitrite and nitrate) in the brain and serum did not show any significant differences among the control and treated groups. Stress affected the acetylcholine esterase activity at CNS and peripheral levels. Serum acetylcholine esterase activity may be considered as a marker of stress in Japanese quails.

(restraint stress for 30 min) and S 2 (restrained for 60 min) reduced compared to controls thoughout the trial period. The formation of abdominal fat pad enhanced in S1 and S2 groups, about 58% increase in fat pad weight was observed in S2. There was a significant increase in the lipid fractions (phospholipid, triglyceride and cholesterol) of muscle and liver in groups S1 and S2. Similar pattern was also observed in serum of stressed groups. However, HDL-cholesterol level decreased significantly in S2 whereas levels of LDL-cholesterol drastically increased in this group. Besides, elevation of serum glucose and uric acid was noticed in S2 group. Chronic repetitive stress resulted in reduced feed intake, growth retardation and increased fat deposition in abdomen, breast muscles and liver. It also caused a deleterious alteration in the composition of serum lipoproteins in broilers.

# Yak

Efforts were made to develop and validate a direct, simple and highly sensitive enzyme immunoassay on microtiter plates using the biotin-streptavidin amplification system and the second antibody coating for GH determination in yak plasma. Time of ovulation in relation to estrus and LH peak was determined in yaks and estrus was successfully synchronized in yak, using  $PGF_2\alpha$  and GnRH. A positive correlation in breeding and non-breeding seasons between circadian changes in plasma prolactin and melatonin concentrations were recorded in these animals.

The birth weight of the yak calves is significantly affected by the month of calving. The maximum birth weight was noticed in June and it declined thereafter. Maximum number of yaks came to heat from October to January. Castration had no adverse effect on the body weight gain, dry matter intake or nutrient digestibility of growing male yaks.

Embryo transfer technology in yak: The pioneering work

in yak ETT was initiated which indicated, the prospect of implementing ETT for *ex-situ* conservation and for augmenting reproductive efficiency in yak. Three embryos were successfully collected non-surgically from the donor yak cow, and were transferred to a recipient.

#### Mithun

Endocrine control of estrus behaviour in mithun: Typical estrus signs of cattle like vaginal mucous discharge, homosexual interaction, reduced feed intake, frequent urination and vocalisation were not observed in mithun. The visible signs recorded during study were slight restlessness, reddening and swelling of vulva and standing to be mounted. Average duration of estrus and standing heat were recorded as 67.2±7.2 and 10.5±2.7 hr, respectively. In 40% experimental animals, standing heat was absent. Nearly significant close associations were observed between estrous intensity score (EIS) and basal concentrations of plasma estradiol 17 ß (E2) and progesterone (P4). Expression of estrus in mithun is silent and for behavioural expression of estrus, progresterone (P4) level was instrumental in relation to E2 concentration of entire cycle. The low behavioural expression of estrus in mithun was probably because of overall low P4 concentration in entire estrous cycle. Highly sensitive enzymeimmunoassays for GH and LH using the biotin-streptavidinperoxidase amplification system were validated in blood plasma of mithun on second antibody coated microtitre plates.

**Absorption efficiency of colostral immunoglobulin:** Immunoglobulin fractions (IgG, IgG<sub>1</sub>, IgG<sub>2</sub>, IgA and IgM) were estimated. The serum total immunoglobulin concentration (mg/ml) recorded at 0 hr (before colostrum feeding) was  $4.28 \pm 1.26$ . After colostrum feeding, the total serum immunoglobulin concentration incurred at 6 hr (22.72 ± 6.49), attained peak concentration at 24 hr (40.93 ± 8.67) and then decreased at 72 hr (30.23 ± 6.60).

**Progesterone estimation:** A simple, reliable and highly sensitive radioimmunoassay (RIA) was developed and validated for progesterone determination in mithun plasma.

# Milk and milk products technology

**Dairy ghee** attenuates chemical induced mammary and gastrointestinal carcinogenesis. Dairy ghee attenuates diet-induced hypercholesterolaemia, and cow ghee is more efficacious. It also stimulates body immune system, improves antioxidative status, and increases tissue levels of conjugated linoleic acid (CLA). Anticarcinogenic, antimicrobial immunomodulatory, hypocholesterolemic, antioxidative and ACE inhibitory attributes of probiotic *dahi* and *lassi* were established. A process was developed



- Techniques developed for commercial manufacturer and improving shelf-life of several milk products
- Churpi and flavoured whey (byproduct of churpi) were developed from yak milk
- HACCP design for ready-to-eat sausage was prepared
- BPE @ 0.2% enhanced shelf-life of chicken-skin cutlet
- Higher bacterial counts in egg observed at retail outlets than at wholesale market or poultry farm
- Residual lead was highest in liver followed by egg and muscle
- Choudhery charkha was used to improve quality of coarse and fine yak wool
- Modified hand knitting technique improved 200% value of yak wool product

for manufacture of long-life *dalia* dessert in ready-to-serve (RTS) form using a rotary sterilizer. The improved processes for kheer mix and rabri were developed. Process for a lassi-like beverage was standardized using rennet whey. A technology was developed for packaging and storage of *dahi* in eco-friendly shellac coated earthen pots. Processes were developed for instant rasmalai mix and instant basundi mix. The technology was developed for improving the quality of cow milk *khoa* by adding WPC prepared by ultrafiltration technique. Other technologies were developed for the production of good quality *chakka, shrikhand* and chhana and chhana based sweets, rasogolla and savswah with higher yield and higher milk solids recovery using ultrafiltration process. Flavoured milk beverage was standardized at the SRS, Bangalore, for the production of a carbonated beverage. The injection of carbon dioxide into the milk beverage decreased pH of the product. Presence of dissolved carbon dioxide in the beverage did inhibit the growth of microbes in the samples. Upgraded technology for preparation of *kalan*, a traditional

#### Goat milk—a future health food

Goat milk is known for its nutritional and medicinal qualities. Goat milk may be designed to best fit consumer need by genetic manipulation. Genotyping at CSN1S1 locus can be carried out in Indian goat breeds for improving both quality and quantity of milk. The allelic distributions of the  $\alpha$ s<sub>1</sub>-casein in the Indian goats were quite different from European goats. SDS-PAGE and DNA analysis revealed that majority of Indian goat breeds and non-descript goats were carrying A, B, C, D, E, F allele at  $\alpha$ s,-casein locus. However A, B alleles were observed in highest proportion in Indian goat breeds. F allele was observed in Beetal, Marwari, Chegu and non-descript goats of Madhya Pradesh region and in less than 1% of population. The gene frequency of allele A in Indian goats varies from 0.68 and 1.00 and allele B varies from 0.098 and 0.23. Milk proteome analysis of different breeds was carried out by 2-D gel electrophoresis.

concentrated dahi-based product from Kerala, was developed at the SRS, Bangalore. Packaging of this product in flexible pouches followed by controlled heating (below 100°C) increased the shelflife to more than 3 months. Retort processing was more useful for commercializing this product. A prototype refrigerated twin-screw forming system (plasticizer) was developed to manufacture recombined butter (Butter-G) from ghee in a continuous manner. The unit, producing 100 kg butter/hr, can be scaled-up for higher capacities. The equipment is simple and hygienically designed. Good quality **burfee** was produced through engineering and technological modification of thin-film scraped surface heat exchanger. A third stage SSHE with better operational control, and sugar dosing mechanism was incorporated in the continuous khoa-making machine. A number of presentable prototype equipment to produce indigenous milk products are available for regular demonstration to the end users. The mechanized process for kunda manufacture reduced time of manufacture and energy consumption. A protein fraction having applicability

#### Low cholesterol ghee developed

Taking lead amongst the dairy research, scientists working on preparation of low-cholesterol food products world over, a process was developed for preparation of low-cholesterol ghee with 90% of dietary cholesterol removed in the process, at the NDRI, Karnal. The low-cholesterol ghee prepared by newly developed process would prove to be a boon to cholesterol conscious consumers. The process developed does not have any limitations in terms of prohibitive cost and quantitative/ qualitative loss of milk fat, and perfectly meets the standard specifications of ghee under Prevention of Food Adulteration and AGMARK rules. The process developed at this institute is presently for laboratory scale. A patent is being filed for the newly developed process.

in preparation of **infant formula** was obtained. **A plasmid based food-grade cloning** and expression vector host system was developed for *Lactobacilli* for first time in India. Food grade bacteriocin based biopreservative formulation was developed and found effective in enhancing shelf life of *paneer* and *khoa*. A laboratory scale process for **calcium fortification of milk** was developed. Thermal stability and calcium bioavailability of such milk was studied. A laboratory scale process for preparation of coconut filled *Gouda* cheese was developed. The applicability of estimation methods of lactic acid in milk, in presence of additives, was assessed. There was no interference of neutralizers and preservatives in estimation of lactic acid by either AOAC or IDF method. The Bieber's test heitherto used for detection of almond oil adulteration with kernel oil, was modified to detect adulteration of ghee with 5-10% vegetable oils. Three-phase-partitioning (TPP)



# Preservation of chicken skin-meat cutlets by natural preservatives

Processing of chicken skin-meat cutlets with 0.2% BPE enhanced the shelf-life of the product up to 17 days under refrigeration  $(4\pm1^\circ\text{C})$  and 32 days under frozen (-18°C) storage conditions.

was applied to fractionate whey proteins.

**Mechanized rasogolla manufacturing plant:** Rasogolla, one of the most popular dairy sweets of the Indian subcontinent, is gaining popularity in the other parts of the world with the spread of the Indian diaspora around the globe. The process for rasogolla manufacture was mechanized by inventing a continuous *chhana* ball forming system. In the final step, the chhana balls are cooked in sugar syrup for obtaining spongy *rasogolla*. For this purpose, a continuous cooker has been developed, where optimum cooking conditions such are concentration of sugar syrup and heat flux are maintained at optimum level to obtain the desirable qualities of spongy *rasogolla*.

**Churpi** and butter making: The process control points of *churpi* making practiced by yak farmers in their traditional way were investigated. The performance of traditional process equipment (butter churn, *churpi* making basket, etc.) was determined. The standardized method provides more yield of *churpi* and butter per kg of milk and the products are more hygienic with longer keeping quality.

**Utilization of whey—a byproduct of churpi making:** The whey has been converted into salted/sweetened health drink with pineapple and jeera flavours. The process technology is simple and can be adapted at farmer's level on small scale. The consumers and sensory panelists evaluated the products as 'excellent' to 'good'. The keeping quality of the whey health drink (salted/ sweetened) has been 2 weeks at refrigerated temperature of  $4-5^{\circ}$ C.

# Meat and meat products technology

*Sheep:* Mutton samples were analyzed for DDT, Aldrin, Endrin, Dieldrin, Endosulfan, Heptochlor and BHC by GLC with ECD. None of the above compound was detected.

**Goat:** Old Sirohi goats had average carcass weight of 23.98 kg and dressing percentage of 44.85%. The yield of spice extract stock solution (SES) was  $200\pm20$  ml for a 600 w/v (100g spice mixture + 500 ml distilled water) mixture. Addition of 75% spice extract solution in the formulation was rated best by the semi-trained panelists. Combination of hurdles had definite influence on microbiological characteristics of stored sausages. Microbiological status of the stored sausages suggests that the product prepared by combined treatment could be stored for 30 days. Coliform counts

were not detected throughout the storage. Average carcass weight was 15.12 kg for male Muzaffarnagari lambs and 13.30 kg for female lambs and the dressing percentages were 43.29 and 44.28% respectively. Average carcass weight of the Barbari goats was 11.37 kg and the dressing percentage was 44.94. HACCP design for the production of ready-to-eat cooked sausage was prepared and presented.

**Rabbit:** The per cent inedible offal yield was higher in males (21.86%) than that of females, and was higher in grower (25.30%) compared to adult stock (21.80%).

**Yak:** Meat samples were collected from Nyukmadung farm and local areas of Dirang, West Kameng district of Arunachal Pradesh. Vitamin B., B., and vitamin E estimations were completed.

*Pig:* Formulations for low salt, low fat, medium fibre pork meat balls were developed.

**Poultry:** Retort processing of chicken chunkalona from culled hen meat: A retort process was standardized for the shelf-life extension of value-added premium product from the less desirable tough meat of spent hen. Retort-processing of chicken chunkalona containing spent (culled) hen meat (SHM) (60%), skin-gizzard-heart (15%) in natural proportion and marinated broiler meat chunks (25%) in combination with non-meat binders and extenders yielded a delicious, restructured, shelf-stable product. Retort- processed (1kg/cm<sup>2</sup>; 30 min) product containing either 200 ppm  $\alpha$ -tocopherol acetate or 200 ppm BHA and packaged in retortable, flexible laminated (aluminum foil-PP) pouches could be safely stored up to 2 weeks and 2 months at mean ambient (25°C; 62% RH) and refrigeration temperature (5±1°C; 80–85% RH) respectively.

Development of value-added egg product: Process of preparing batter-breaded egg rings, a delicious snack food prepared from whole egg liquid, was standardized. The rings battered in 25% rice flour + 15% black gram flour coating was most acceptable and had refrigerated  $(4\pm1^{\circ}C)$  shelf-life of 12 days in aerobic packaging with satisfactory microbiological and sensory quality. The cost of formulating 1 kg egg rings was estimated to be Rs 39.04.

Residues of harmful substances in poultry products: The

# Surveillance of bacteriological quality of chicken eggs

Occurrence of pathogens such as Salmonella and Escherichia coli in chicken eggs collected from poultry farms located in Uttaranchal region and local markets of Bareilly was 2.90% and 12.36%, respectively. Molecular technique of detection of Salmonella from chicken eggs using his j gene was standardized. Higher bacterial counts (total mesophilic count) were found in eggs collected from retail outlets than those collected from wholesale market and fresh eggs from poultry farms.



#### Camel milk lotion/cream

Organic camel milk lotion was prepared by adding preservatives. Initial evaluation of lotion indicated positive impact on imparting shine to the skin without any oiliness and also improved the roughness of the skin.

residual level of lead was highest in liver (0.35 ppm) followed by egg (0.3 ppm) and muscle (0.2 ppm) in poultry. The levels of arsenic in egg, muscle and liver were recorded as 0.35, 0.25 and 0.35 ppm respectively. In muscle the level of BHC ranged from 0.05-0.1 ppm, whereas, in liver and adipose fat it ranged from 0.1 to 0.2 ppm and 0.2–0.5 ppm, respectively. Per cent occurrence of BHC was higher in adipose tissue (33%) during August–November. The residual concentration of DDT was 0.08–0.2 ppm in muscle, 0.15–0.25 ppm in liver and 0.2–0.3 ppm in adipose tissues. Malathion and aflatoxin B1 levels were very low (0.05–1.0 ppm).

Processing technologies and formulations for use of vegetables, eggs and byproducts in meat products and high valued meat bites – chicken, mutton and combination were developed. A process was developed for the production of chicken soup from deboned frames, as a byproduct. Value added products of nutritional merit from chicken necks with bone and buffalo offal meats (tripe, heart and head meat) were developed.

# Wool

**Quality analysis of Magra sheep wool:** At the ARC Bikaner, study carried out on Magra wool samples of spring clip of 1-year old animals to find out the lustre through subjective means, revealed overall rating of 3.75 on 5 grade scale, which can be termed as very good luster.

# Knitwear from corase yak wool

It is very difficult to make value added handicraft from coarse yak wool. Hand knitting technique with minor modifications made it possible to make pullovers, caps, hand gloves, etc using corase wool span.. The value addition was to the tune of 200%.



*Coarse yak wool spinning using Choudhery Charkha:* The traditional hand spinners are in operation for converting fine yak wool yarn (single and double ply thread). It is very difficult to make thread from coarse yak wool using traditional spinners. These are also very slow and only few old crafts men can operate and fabricate them. To improve the speed and quality of coarse and fine yak wool yarn Choudhery Charkha was introduced. The Charkha was successfully adapted for both coarse and fine wool yarn making and tribal ladies were trained in Charkha operation. In LPT Unit 5–6 kg yarn was made using Charkha.