

# Livestock Management

## LIVESTOCK PRODUCTION

### Strategic supplementation of macronutrients:

Supplementation of locally available energy rich feed ingredient (*ragi* grain) to lactating cows increased milk production (1.0–1.5 kg) and milk fat (0.25–0.5%), and reduced milk urea nitrogen (MUN) and thereby increased cost-benefit ratio.

Strategic supplementation of maize grain and protected fat improved or maintained milk production in 60% of lactating cows. Supplementation of mixture of barley and mustard cake (1:1) to growing heifers on wheat straw based rations showed that the mixture of protein and energy was a better supplement than either barley or mustard cake alone. Mixture of maize and niger cake supplementation increased milk production by 0.5 kg/day in lactating animals. A limited supplementation of wheat bran (250 g/head/day) and common salt (20 g/head/day) or barley (250 g/head/day) and area-specific mineral mixture (20 g/head/day) improved the body weight gain in growing male calves under grazing condition.

**Area-specific mineral mixture:** Supplementation of area-specific mineral mixture to lactating cows improved feed intake, milk yield, milk composition, efficiency of feed utilization and

reproductive performance (like early exhibition of post partum estrus, higher conception rate with single insemination). Increase in daily milk yield was 0.5–1.0 kg/animal/day and fat 0.3 – 0.5%. Nutrient utilization, and mineral status as well as general health of the animals improved on supplementing the area-specific mineral mixture.

**Bioavailability of micronutrients:** Tissue level utilization of Cu and Zn was more in ewes supplemented with organic than inorganic sources of Cu and Zn. Supplementation of organic mineral at lower levels as compared to inorganic minerals showed similar performance with respect to body weight gain, absorption and retention of minerals in male calves. Supplementation of Zn and Cu from organic sources was more effective in inducing estrus in anoestrus crossbred cows.

**Reproduction:** Flushing of 6 donor Frieswal cows superovulated with 300 mg follotropin-V resulted into recovery of 24 embryos, out of which 10 were morulae, 2 blastocysts, 7 degenerated and 3 unfertilized ova. Transfer of 7 good embryos

### Management information system for *Gaushalas*

A database integrating data on different aspects of *Gaushalas* was developed. This database on 105 *Gaushalas* of Haryana, has information on history, breed-wise herd strength, assets inventory, budget and expenditure, entrepreneurial efforts made by *Gaushala* management, linkages with other agencies, breed improvement programmes, maintenance of milk recording and pedigree of animals, common diseases, constraints and manpower employed.

### Evaluation of unconventional feed resources

Costlier conventional cake like soybean meal was replaced completely by canola quality rapeseed-mustard cake (RMC) as a protein source in the diet of young calves. Though glucosinolates (anti-nutritional factor) of RMC appear to have no adverse effect on nutrient utilization, its feeding significantly reduces the palatability of concentrate and overall performance of animals. Overnight soaking of RMC in water drastically reduced the level of glucosinolates, however, feeding of water soaked RMC did not improve animal performance. Lime treatment of castor seed reduced (58%) its ricin (anti-nutritional factor) content. Feeding of raw and lime-treated castor seed cake did not affect the nutrient utilization, biochemical profiles of rumen and blood plasma as well as histology of vital organs.

to 7 recipients resulted into 3 pregnancies, confirmed on rectal palpation.

Ejaculates (1–134 ejaculates/bull) were collected from 87 Frieswal bulls. Thirty-one bulls produced 235,011 frozen semen doses (7,581/bull/year). The overall mean abnormal head, mid piece, tail and total abnormal sperms in seminal ejaculates of Frieswal bulls were 10.27, 4.93, 13.93 and 29.13%, respectively. A large number of Frieswal bulls (63.41%) produced non-freezable quality semen with a very high occurrence of abnormal spermatozoa in the ejaculates.

**Methane emissions from manure of Indian livestock:** The methane emission from fresh dung of indigenous Tharparker breed of cattle was 8.22 mg/day and in Sahiwal was 9.06 mg/day. Emission of methane was higher, about two-folds, in crossbred cattle (15.06 mg/day in Karan Fries) and Murrah buffaloes (14.45 mg/day) compared to zebu cattle. Methane emission from fresh dung on dry matter basis was low in zebu cattle as compared to crossbred cattle. The annual methane emission estimates from dung of Tharparker was 3.00 kg, Sahiwal 3.3 kg, Karan Fries 5.4 kg, and in Murrah buffaloes 5.2 kg. Methane emission on storage increased after 8–10 hr of deposition, and emissions were at peak in about 8–10 days after deposition. Diurnal fluctuations in methane emission were also observed and were related to ambient temperature rise. Emission of methane was at peak during afternoon and thereafter, it declined during night due to low fermentation activity and decline in ambient temperature.

#### Buffalo

Isolates of sulphate reducing bacteria (SRBs) were isolated — 7 from rumen of buffaloes and 7 from faeces of buffaloes. Significant increase in digestion and gas production was observed from wheat straw when some of the SRB isolates were added to buffalo rumen liquor inoculum.

High energy fed buffaloes, showed lower ovarian activity and gravid status, indicating adverse effect of abrupt change in ration. Field level demonstration indicated that replacement of cotton seed cake with cheaper mustard cake in prevailing feeding practices of cattle and buffaloes saved Rs 90/animal/month without compromising on milk production.

**Bioavailability of micronutrients:** Concentrations of Zn and Cu in fodders cultivated in various soils of district Meerut were within normal range. The Zn, Cu and Co content of water collected from different areas ranged between 0.059 and 0.2, 0.039 and 0.048 and 0.042 and 0.134 ppm, respectively. Groundnut cake and gram *chuni* had higher Zn than the reported values. The concentration of Zn, Cu and Co in blood plasma

## SUCCESS STORY

### 'GARIMA'

#### World's Second Cloned Buffalo Calf

World's second cloned buffalo calf produced through the new and advanced 'Hand-guided cloning technique', was born at the NDRI, Karnal on 6 June 2009. The scientists of NDRI had developed this land mark technique, and had also produced the world's first cloned buffalo calf on 6 February 2009. This cloned buffalo calf was different from the first one, as in this case the used donor cell was taken from a foetus. However, in world's first cloned buffalo calf, produced earlier through this technique at the NDRI, the donor cell was taken from the ear of a newborn calf. In other words, this buffalo calf was a clone of foetus that had not even seen the light of the day. The hand-guided cloning technique is an advanced modification of the conventional cloning technique.



of cattle and buffaloes were optimum and within the normal range of 1.98–2.19, 1.36–1.63 and 1.23–1.85 ppm.

**Enhancing conjugated linoleic acid (CLA) in milk:** Green fodder feeding increased milk CLA in cows and buffaloes. Further, it increased up to 310% in *ghee* prepared by indigenous method. Feeding CLA during the exposure of rats on carcinogen, enhanced liver and RBC lysate antioxidant enzyme activities (catalase SOD and GST), reduced liver lipid peroxidation and mammary cancer risk (37%). Milk having high CLA (19.50 mg/g fat) may be consumed @ 3 litre/day (provide 3 g CLA) to have a protection against cancer; however, normal milk required more than 13 litre to achieve this protection.

**Sperm quality:** CASA parameters for sperm motility, progressive motility and rapid motility in higher, medium and lower fertility groups of bulls revealed that these parameters did not vary significantly between medium and high fertility group, whereas, differed significantly from the lower fertility group; thus enabling identification of poor fertility bulls.

The study on requirement of minimum number of sperms in an insemination dose to make more efficient use of quality semen from superior bulls,

revealed that conception rate with 25 and 15 million dose of spermatozoa is similar. Pregnancy could also be achieved with 5 and 3 million dose of spermatozoa with conception rate of nearly 20%.

### Sheep

Grazing resources constitute major source of forage for sheep under existing system of extensive management. Undeveloped lands yield 9–14q of forage during different months of a year. The dry matter (DM) intake in dry ewes ranged from 672 to 1,251 g, and digestible crude protein (DCP) 115.11 g and metabolizable energy (ME) 98.82 kJ. Sheep were unable to meet DM and DCP requirements except energy on undeveloped lands of semi-arid region of Rajasthan, and need strategic supplementation of the limiting nutrients for enhancing production and reproduction.

*Prosopis juliflora* (*Vilayati babool*) is widely found across the country and poorly utilized by animals owing to higher toxins. Methanol treatment was effective in ameliorating toxic effect but is relatively costlier.

Commercially available microbial feed additives containing *Saccharomyces cerevisiae* + *Lactobacillus sporogenes* enhanced growth by 12.0% and feed intake by 11.6% in fattening lambs for mutton production.

Fat incorporation up to 5.00% level in the ration of lamb increased weight gain, improved feed conversion ratio, maintained carcass quality within acceptable norms, reduced feeding cost/kg weight gain, improved benefit: cost ratio, and was advantageous in lamb feeding for mutton production.

Malpura male lambs reared under intensive management system produced good quality semen at 9–12 months of age for breeding and AI purposes. The concentrate mixture feeding @ 1.5% of body weights during autumn increased the lambing rate to 91.6% compared to 72.7% in non-supplemented animals. The ovagen in conjunction with progesterone increased lambing percentage to 133% compared to 88.8% under routine practice in Malpura sheep. Daily milk yield of Patanwadi sheep was 783 g and of Malpura 515 g under optimum feeding condition.

#### Better lamb weight at young age

Most of the farmers sold their male lambs at 3–4 months of age at body weights of 12–13 kg mainly due to immediate financial requirements, feed and fodder scarcity in the region and also due to fear of mortality losses. The supplementation of concentrate mixture @ 1.5% body weight during post-weaning stages improved body weights by 24.18% and farmers fetched 25–33% more price in the market.

### Goat

#### Oilseed cake containing diet for Barbari goats:

Goats fed rations containing cotton seed cake and linseed cake in the ratio of 50:50 showed better results in terms of — weight of pregnant Barbari does just before kidding and weight of does just after kidding. Higher ratio of linseed cake in the diet of does was more effective for higher milk yield and subsequently higher body weight of kids up to 14 weeks of age. Diet containing cotton seed and linseed cakes in the ratio of 50:50 was more effective in pregnant and lactating Barbari goats and on the growth performance in kids.

#### Complete pelleted feed

Complete pelleted feed with concentrate mixture: roughage ratio of 40: 60 could be used for goat production without affecting any carcass traits and meat quality. This complete pelleted feed is economical also.



**Post-thaw seminal parameters:** Ejaculates from adult Jamunapari bucks (3–5-year-old) were prepared by conventional method of freezing and the results showed that 10% egg yolk and 4 hr of equilibration period was the best combination for semen freezing.

**Effects of antioxidants on liquid storage of buck semen:** Ejaculates from adult Sirohi bucks,

#### Reducing the post-partum interval

Indigenously prepared sponges/injections with hormones (eCG, PMSG, GnRH) were used in 16 Jamunapari goats and they were regularly checked for oestrus at 12 hr interval by an aproned buck for 30 minutes. Oestrus response and conception rate following the use of intra-vaginal pessaries in two groups of Jamunapari goats are as follows:

Treatment groups	Oestrus response (%)	Onset of oestrus (hr)	Duration of oestrus (hr)	conception rate (%)
<b>Group 1</b>	5/4 (80%)	21.00 ±10.24	24.00 ±12.96	75
<b>Group 2</b>	9/9 (100%)	34.66 ±5.81	22.66 ±2.40	77.77

prepared at various levels of different antioxidants revealed that semen extended to the diluents having antioxidants (3 mM vitamin C, 1.5 mM vitamin E, 5 mM glutathione oxidized and 5 mM glutathione reduced respectively) can be stored up to 96 hr at refrigeration temperature.

### Camel

**Nutrition:** Camels supplemented with 2 kg concentrate mixture and 50 g mineral mixture/day on cluster bean straw-based diet showed improvement in milk production by 0.74 kg/day. The camel calves obtained from the supplemented dam showed better growth rate than the calves from unsupplemented grazing animals.

**Lana seed diet:** Lana (*Haloxylon salicornicum*) seed feeding to camel revealed that camels preferred these seeds in arid region and showed fairly good digestibility; and it can safely replace the conventional concentrate feed.

### Yak

**Trace mineral supplementation:** Supplementation of area-specific mineral mixture (Zn, Cu, Co and Mn in the ratio of 40:20:2:1) to yak improved growth rate as well as milk yield of animals. It increased conception and fertility of yak and birth weight of yak calves. A higher monthly body weight gain of  $15.17 \pm 2.05$  kg was recorded by feeding complete feed block as compared to  $5.07 \pm 0.87$  kg in traditional feeding.

**Effect of copper supplementation on reproductive traits of yaks:** The soil, feeds and fodders of the yak rearing hilly tract of Arunachal Pradesh are deficient particularly in copper. Yaks are genetically susceptible to copper deficiency. Supplementation of basal diet of yaks with copper sulphate (200 mg/animal/day) effectively enhanced the reproductive performance of animals as the number of animals exhibiting estrous increased significantly from 39.8% to 100%. The number of service per conception decreased to 1.5/animal/conception from 3.5–4/animal/conception. Postpartum involution period decreased significantly (60–90 days from 1 to 1.5 year) after supplementation.

**Deep freezing of yak semen:** A protocol for deep freezing of yak semen was developed. Suitable semen extenders for deep freezing and refrigerated storage (at 5°C) were also evolved. For *in-vitro* production of yak and its hybrid embryos more than 50 oocytes were recovered from the yak ovaries within 2 hr of animal death. The quality oocytes were further cryopreserved by vitrification.

**Adaptability of yaks:** Yaks do not experience cold stress but are susceptible to heat stress during summer at an altitude of 2,750 msl. Temperature humidity index (THI) was suitably modified for

yaks according to altitude and environmental conditions.

### Diurnal variations on physiological responses:

The study on diurnal variations in the physiological responses of yak calves, adult bulls and lactating cow revealed that the physiological responses were at minimum in the early morning and maximum during late afternoon. The physiological responses of calves were significantly higher than adult bulls and lactating yak cows during late afternoon. In all yaks physiological responses were significantly higher during summer compared to winter.

**Environmental effect on female yaks:** The environmental conditions influence the reproductive traits of female yaks. The breeding season starts in July and reaches its peak from September to November at altitudes of 2,750 m above msl. The exhibited estrous and conception rate were highest from September to November.

### Mithun

### Performance of mithun and Tho-Tho cattle

**on tree leaves based ration:** Mithun, a unique animal of NEH region of India, has lots of similarity with Tho-Tho cattle, which are inhabitants of Nagaland. Mithun and Tho-Tho cattle fed on mixed tree leaves/shrubs (*Quercus polystachya*, *Dillenia pentagyna*, *Lagerstroemia* spp and *Ficus hirta*) with paddy straw (2:1 ratio on fresh basis) along

### Draught capability

Mithun bulls were trained for work to assess their draught capability. Adult bulls were not much obedient and difficult for imparting training for work. Later 2 young bulls (less than 2 years of age and body weight ~140 kg) were trained successfully for work. The preliminary approximate estimation indicated that average draught power of a pair of mithun bulls was 0.60 KW.



with concentrate mixture revealed that mithun attained ADG of 507.8 g with DMI of 6.59 kg whereas cattle attained ADG of 392.8 g with DMI of 5.88 kg/day. Overall growth performance was statistically higher in mithun than that in cattle. Weight of the inedible organs like head, limbs and tail weight were more in mithun signifying a higher body sized animal in mithun than cattle. Mithun had higher dressing percentage than that of cattle. There was positive relationship of carcass weight with rib eye in both mithun and cattle. However, there was positive relationship of rib eye area with fat thickness in cattle whereas the relationship was negative in mithun.

## Pig

**Hormonal regulation of fatty acid synthesis and desaturation:** The exogenous growth hormone (GH) and insulin administration increased the body weight gain in grower pigs. Exogenous insulin increased serum GH, insulin, leptin and BUN while decreased serum triglyceride levels. Exogenous GH increased serum GH, insulin, triglycerides, glucose, and decreased BUN levels. The growth hormone decreased the expression of lipogenic genes fatty acid synthase (FAS) and stearoyl CoA desaturase (SCD) under both in *vivo* and *in vitro* conditions in porcine subcutaneous adipose tissue. The leptin under *in vitro* conditions decreases mRNA abundance of both FAS and SCD genes. The insulin increases expression of sterol regulatory element binding protein 1 c (SREBP-1 c), peroxisome proliferator activated receptor  $\gamma$  (PPAR  $\gamma$ ) and CCAAT enhancer binding protein  $\beta$  (CEBP  $\beta$ ) genes suggesting that insulin may activate lipogenesis and adipogenesis in adipose tissue.

**Preservation of boar semen:** Methods were standardized for the preservation of boar semen for carrying out artificial insemination (AI) in pigs. Boars of different strains and breeds were trained for semen collection. Gloves hand method of semen collection was standardized and evaluated for semen volume, sperm motility, percentage of live and dead spermatozoa and concentration of spermatozoa.

## Poultry

**Antioxidant based feed formulation and herbal drug:** An antioxidant (vitamin E) with a medium dose (300 mg/kg feed) in combination with liver stimulant (5 g/kg feed) was found detrimental for egg production in birds. A proper combination (dose) of liver stimulant and antioxidant is to be given to the hen as these combinations are highly interactive and may cause reduction in egg production.

**Effects of moulting by feed withdrawal and mineral induced method:** After 14 days of forced

### Melatonin precursor supplementation alleviated aflatoxicosis

Aflatoxin inclusion in the feed @ 0.5 ppm caused significant reduction on the performance of broilers. Supplementation of melatonin (40 ppm-20 mg/kg bw through IP route and remaining half through feed @ 20 ppm) and its precursor (tryptophan-250 ppm) or toxin binder (250 ppm) numerically improved the performance when offered individually. However, the combination of melatonin and toxin binder significantly improved the performance. Combination of melatonin @ 40 ppm and toxin binder @ 250 ppm alleviated adverse effects of aflatoxicosis in broilers.

moulting by feed withdrawal (FW), high zinc (ZnF) and aluminium feeding (AF), significantly reduced body weight in FW (25%) followed by ZnF (24%) and least in AF group. Caspases 2, 3 and 6 appeared to be the key players in the regression of ovary under moulting in both FW and ZnF treatment groups. Zinc induced apoptosis of hen's ovaries through an intrinsic mechanism. Apoptotic gene p53 appears to play a major role among the mitochondrial apoptotic genes in the regression of magnum. IFN  $\gamma$  gene was upregulated in uterine tissues of hens moulted by either FW or ZnF indicating its major role in the uterine regression during moulting in White Leghorn hens.

**Trace mineral bio-availability in broilers is age related:** Trace mineral (Zn, Mn, Cu, Fe and Se) uptake by broiler chicken as determined by their concentration in tissues (bone and liver) revealed considerable influence of age on mineral retention. The concentration of Zn (354–451 ppm), Mn (16.4–18.6 ppm), Cu (10.5–11.2 ppm) and Fe (254–321 ppm) in tibia significantly declined with the advancement of age from 2 to 4 and 6 weeks, implying higher need for these minerals during early period of growth. The same trend was also noticed with the uptake of minerals by hepatic tissue. In contrast, Se retention was more in tissues during the later period of growth (4 and 6 weeks) than that in the second week. The biological need of trace minerals was not the same in broilers for the entire 6-week period and supplemental levels are to be optimized based on age related needs.

**Early post-hatch nutrition in broilers:** Single intubation of starch, soybean oil, starch + casein, starch + soybean oil or starch + casein + soybean oil into the crop of broiler chicks immediately after hatch produced positive long-term effect on body weight of broilers. However, casein alone or in combination with soybean oil did not prove to be advantageous compared to the above listed combinations. The combination of carbohydrate

### **AICRP on Improvement of Feed Resources and Nutrient Utilization in Raising Animal Production**

- Supplementation of bypass nutrients in the form of formaldehyde treated *guar* meal/rape seed meal and calcium salts of palm oil/rice bran oil to lactating buffaloes and cattle improved milk yield (1–2 kg/day/animal), reproductive efficiency and sustained peak milk yield for longer period
- Strategic supplementation of limiting macronutrients with locally available feeds and concentrate mixture to medium producing dairy cattle under field conditions increased milk yield (1–1.5 kg/day/animal), milk composition, and reproductive efficiency.
- Feeding of complete feed blocks or pellets to dairy cattle and buffaloes under grazing system improved milk yield (1.0 kg/day/animal), fat content and maintained the persistency of production. This approach is most suited for scarcity situations under field conditions.
- Areca (*Areca catechu*) sheath, a by-product of areca plantation proved a valuable roughage source for dairy animals, were superior to paddy straw with respect to nutrient utilization, mineral and lignin content.
- Supplementary feeding of concentrate mixture to kids of Jamunapari and Sirohi goats and lambs of Malpura sheep @ 1.5% of their body weight showed better growth and health, and attained marketable weight earlier than unsupplemented kids/lambs.
- Supplementation of copper and zinc in chelated form to lambs of Rambouillet sheep under intensive feeding system improved their antioxidant and immune status.
- Supplementation of protein through soybean meal along with other ingredients showed better growth and health in pigs. Supplementary feeding of mineral-vitamins-amino acid mixture to breeding and lactating sows resulted in increased weight of piglets, weaning percentage and better profit.
- Feeding of feed blocks comprising local feeds/fodders, molasses and minerals were advantageous to yak during winter in high altitude.
- Enriching and ensiling of sugarcane tops improved its nutritive value for feeding to cattle.
- Mineral profiles of soil, feeds/fodders and animals in Tripura and Sikkim revealed that calcium, phosphorous, copper, zinc and manganese were the most limiting minerals for livestock production in these regions.

and fat yielded better results than that of fat + protein. The chicks could utilize carbohydrate better than fat during initial post-hatch period.

**Organic acid in broiler diet:** Butyric acid @ 0.4% in the diet of broiler chicken enhanced body weight gain and feed conversion efficiency and reduced *Escherichia coli* count. Several additional

effects such as stimulated growth of intestinal villi, higher carcass yield and low abdominal fat content were also observed by dietary addition of butyric acid.

**Alternate feed ingredients for Krishibro broiler:** The Krishibro chicks responded similarly to 3-phase and 2-phase feeding regime during 0 to 6 weeks of age. Replacement of maize with dried distillery grains on w/w basis in their diet indicated that dried distillery grains could replace maize with beneficial effects up to 33% level, and higher levels of inclusion were detrimental. Sunflower cake could be used for replacing soybean meal protein up to 50% in the diet of Krishibro.

**Nutrient requirements of Vanaraja chicks:** Replacement of soybean meal with mustard cake depressed performance of *Vanaraja* chicks, and additives betaine, Cu, liver tonic etc. were not useful in amelioration. Dietary supplementation of certain herbal extracts improved feed conversion efficiency and antibody response in normal chicks. Na or Cl concentration in diet beyond 0.2% adversely affected cell-mediated immune response and enhanced tissue oxidation. Soya isoflavin in diet positively influenced tissue oxidation and immune response.

**Pesticide residue in feed and poultry products:** In northern region under study, the residue level of BHC in muscle of chicks ranged from 0.05 to 0.25 ppm whereas in liver, adipose fat, poultry feed and egg it was in the range of 0.02 – 0.1 ppm, 0.1 – 0.2 ppm, 0.15–0.25 ppm and 0.01 – 0.015 ppm, respectively. Samples collected from Haldwani and Lucknow had lower occurrence (8.33%) of residue of BHC than those of Gurgaon and Delhi. The level of DDT was recorded to be 0.03 – 0.1 ppm in muscle, 0.05 – 0.1 ppm in liver, 0.1 – 0.2 ppm in adipose tissues, 0.01–0.02 ppm in egg and 0.1–0.2 ppm in poultry feed. DDT was more (20%) in adipose tissue collected from Delhi market. Like BHC, samples from Haldwani and Lucknow had similar pattern of residue level of DDT. The market samples had relatively higher levels of residues as compared to that of the farm samples but all the samples analyzed had these residues below their MRLs.

**Cloacal foam in quail reproduction:** A study, carried out using perivitelline technique and AI to understand the actual role of foam during natural mating, revealed that foam has a role in sperm transport.

## **LIVESTOCK PROTECTION**

### **Foot-and-mouth disease**

During the year, 511 outbreaks were recorded as against 1,211 outbreaks during the year 2007–

08. Virus could be diagnosed in 376 samples (serotypes O-334, A-26 and Asia1-16). In all the geographical regions, other than central India, serotype O was the most prevalent one. Asia-1 was completely absent in Southern India, whereas type A was absent in the Central and Northern India.

This year, 53 virus isolates including 41 type O, 3 Asia-1 type and 9 A type field isolates were added to the National FMD Virus Repository. At present this national repository contains 1,455 (934, O; 264, Asia -1; 242, A; 15, C) well characterized field isolates.

Molecular epidemiological analysis of 40 serotype O isolates, drawn from 14 states revealed complex genetic diversity of the field isolates in the country, which was not reflected in antigenicity. This year, 'Ind2001' strains re-emerged in Northern and Southern states of the country. The "PanAsia

#### FMD Control Programme

Seven regional FMD centers and the Central FMD Laboratory participated in the sero-monitoring programme. Gradual increase in protective antibody response was observed subsequent to phase 1 vaccination and the titre of the protective antibody level was monitored.

II" strain, which dominated serotype O outbreaks in previous year was restricted to only a few states, while the parental PanAsia strain (PanAsia I) was detected in Bihar, West Bengal and Orissa. In type A, all the isolates clustered within genotype VII and precisely in the VP3<sup>59</sup> deletion group.

#### SUCCESS STORY

##### Foot-and-mouth disease kit (rDIVA-FMD Kit): Development and application

Foot-and-mouth disease (FMD) continues to be a challenge as far as international trade of livestock and their products is concerned. As FMD is endemic, vaccination with inactivated vaccine is the sole mode of control. In such a scenario, it is essentially required to have a DIVA test in place to differentiate virus infected animals from those vaccinated with inactivated FMD vaccine. ELISA was developed at the Central FMD Laboratory, Project Directorate on FMD, Mukteswar, for differentiation of FMD virus infected from vaccinated animals (DIVA). This indigenously developed rDIVA-FMD kit is the first of its kind in the country and was designed as per the OIE approved guidelines and its cost is one-fourth of the commercially imported DIVA kits. Random bovine serum samples (18,326) collected @ 100/district from 234 districts covering 20 different states of the country were tested in DIVA ELISA; this revealed that 28% of the bovine population in the country to be FMD infected during 2008–2009 at confidence level of 95%.

FMD virus typing ELISA kits were manufactured and supplied which ensured uniformity in application and test result across the country.

**FMD reference laboratory:** The institute PD FMD has got the world recognition and is now a member of the Global FAO/OIE Network of FMD Reference Laboratories that constitutes of 10 other FMD laboratories in the world. This institute also participates in global FMD vaccine matching exercise, and is also a member of Global Foot and Mouth Disease Research Alliance (GFRA). Establishment of this international laboratory at Bhubaneswar, with state-of-the-art features of bio-safety and bio-containment (BSL 3+) will facilitate Global participation, and eradication of the disease from South Asia.

#### Animal Disease Monitoring and Surveillance

**Infectious bovine rhinotracheitis:** Six suspected tissue samples were processed for the diagnosis of BoHV-1 infection and all were found positive by PCR using primers specific to the gB (443 bp) partial gene of BoHV-1. Detection of BoHV-1 in semen samples by real time PCR was optimized. Serum samples (4,821) collected from Andhra Pradesh, Gujarat, Jammu and Kashmir, Jharkhand, Karnataka, Maharashtra, Manipur, Orissa, Rajasthan, Tamil Nadu, and West Bengal were subjected to Avidin Biotin ELISA, and out of these 1,423 serum samples were positive for antibodies against BoHV-1.

**Classical swine fever:** Analysis of blood and tissue samples from swine for virus isolation showed at least two populations of subgroup 1.1-viruses involved in the outbreaks during 2006–08 in the country. Sero surveillance of pig serum samples from Andhra Pradesh (377), Karnataka (294), and Kerala (156) indicated that 25% samples were positive for antibodies against CSFV.

**Brucellosis:** Serum samples (948) with the history of abortion, repeat breeding problems and retention of placenta were collected and screened for the presence of anti-*Brucella* antibodies using

#### SUCCESS STORY

##### Development of Indirect ELISA for diagnosis of brucellosis in small ruminants

An indirect-ELISA (i-ELISA) for diagnosis of brucellosis in small ruminants was developed and optimized. Results of i-ELISA were compared with that of RBPT and STAT using sera (398 goats and 604 sheep) of small ruminants and taking CFT as gold standard. RBPT showed the least relative sensitivity in, both, sheep and goats, and I-ELISA could be used as a validated test for diagnosis of brucellosis in small ruminants.

PCR technique. The prevalence of anti-*Brucella* antibodies in cattle ranged between 11.66% and 61.17% (RBPT), 11.66% and 55.29% (STAT) and 39.50% and 75.29% (AB-ELISA). Out of 97 buffalo serum samples tested, 3 (3.09%), 1 (1.03%) and 5 (5.26%) showed positive reaction for the presence of anti-*Brucella* antibodies by RBPT, STAT and AB-ELISA, respectively. An indirect-ELISA (iELISA) was used for screening of brucellosis in swine, and *Brucella abortus* was isolated from pigs, which strengthens the notion that *Brucella* spp infects unnatural hosts also.

**Trypanosomiasis:** Sensitivity and specificity of diagnostic PCR for detection of carrier status of *Trypanosoma evansi* was tested. The primer pair showed 400 bp amplification with genomic DNA isolated from dog, lion and leopard. Detection of viability of *T. evansi* in frozen tissues showed that none of the mice revealed the presence of parasite on blood smear examination. In buffalo blood, the PCR could detect the genomic DNA isolated from buffalo on third day post-infection onwards. Screening of 1,015 serum samples for anti-*N. caninum* antibodies showed that among the sero-positive cattle 63.73% were having the history of reproductive failure whereas among sero-negative cattle only 16.03% had history of reproductive failure.

**Food-borne pathogens:** Food-borne pathogens were isolated and identified, and confirmed by PCR in livestock and livestock products, and an RTPCR assay was also standardized for *Listeria*.

**National Animal Disease Referral Expert System:** NADRES, developed to carry out epidemiological studies, is a web-based interactive

#### Patent application filed

- Recombinant antigen based rapid sero diagnosis of infectious bursal disease. (Patent application No. 491/DEL/2008 dated 03/03/2008)
- Development of a novel bilateral external skeletal fixation device for the management of long bone fractures in large animals (Patent application No. 1964/DEL/08)

programme, created using regression analysis based on databank animal disease trends, disease prevalence, meteorological data, land use data, human demography, soil pattern data and crop production data. It can predict the probability of the occurrence of 10 major livestock diseases in any particular district of the country. The program is available for the general public and can be accessed at [www.nadres.res.in](http://www.nadres.res.in).

#### Gastro-intestinal parasitism

Epizootiological studies on gastro-intestinal parasitism in livestock farms and villages of

different climatic zones showed that in Rajasthan the suitable period for propagation of *Haemonchus contortus* and *Trichostrongylus* spp. is from mid-May to mid-October and from October to mid-February, respectively, in semi-arid zone and from June to mid-September (*H. contortus*) and October to April (*Trichostrongylus* spp.) in arid zone. A software 'FROGIN' was developed for precise prediction and forecasting of haemonchosis in sheep and evaluated in semi arid and arid zone of Rajasthan and anthelmintic intervention was made accordingly to limit the intensity of infection. Genetic improvement of resistance to *Haemonchus contortus* in Malpura sheep showed that magnitude of monthly mean FECs remained lower (15.02% in May to 72.43% in November) in R-line compared to S-line. Similarly, in Avikalin sheep, monthly mean faecal egg count (FECs) ranged from 4.61% (September) to 67.93% (November) lower in R line compared to S line. Keeping in view the level of anthelmintic resistance in *Haemonchus contortus* a mechanism based on conjunctiva colour (targeted selective approach) was developed for further reducing drenching frequency in sheep flocks. Implementation of this approach in sheep flocks reduced anthelmintic use by 70% as only 30% of animals required anthelmintic intervention during wormy season in Rajasthan. Prevalence of GI infection in Tarai of Uttarakhand, in cattle was highest (17.32%) in June, in buffaloes (16.66%) in February, in sheep (100%) and goats (95.31%) in July. In Sikkim, *Haemonchus*, *Bunostomum*, *Nematodirus* and *Oesophagostomum*, spp. helminthic infections were

#### SUCCESS STORY

##### Development of post milking teat dip for prevention of bovine sub-clinical mastitis

An ideal herbal post milking teat dip for prevention of sub-clinical mastitis in lactating dairy animals was developed to reduce the somatic cell count (SCC), total bacterial count (TBC), besides to minimize chemical residues in the milk. The newly developed polyherbal post-milking teat dip was developed using easily available medicinal herbs and its production cost is very low. The ingredient can be easily procured by the farmers and the dairy owners for its preparation. This polyherbal post-milking teat dip was effective in reducing the SCC and TBC in cows.

recorded higher in goats (76.16%) than cattle (31.49%) and yaks (26.66%). *H. contortus* was predominant infection in all the 3 species. Infection rate was higher in high rainfall area (60.7%) than rest of the zones with least infection (41.5%) in Western zone of Tamil Nadu. Immunization trials

## SUCCESS STORY

### Bilateral External Skeletal Fixation Device for Treatment of Long Bone fractures in Large Animals

Management of long bone fractures in large animals is a great challenge to veterinary surgeons. Heavy body weight of animals, angular placement of long bones, and problems associated with postoperative management make the fracture fixation difficult. To address this a simple design involving fixation of bone fragments using transosseous fixation pins was developed which provides rigid fixation of long bone fractures and gives flexibility in the use of maximum number of transfixation pins for tackling compound fractures.

in sheep against *H. contortus* revealed that response of CD4 lymphocytes significantly improved following the priming and booster dose of H11 and H-gal-GP polypeptides.

### Haemorrhagic septicaemia

A biofilm based vaccine was developed against sheep pasteurellosis and was found safe. More than 70 isolates of *Pasteurella multocida* belonging to cattle, buffalo, goat, sheep, pig, duck, poultry and deer were identified and characterized by conventional and molecular tools (PCR, REP-PCR, ERIC-PCR etc.). These isolates were typed as capsular types A, B and D and were sensitive to enrofloxacin, gentamicin, cefotaxime, ceftriaxone and ciprofloxacin antibiotics. The immunodominant plpE gene of *P. multocida* serotypes A, B and D was characterized by cloning and sequencing, and a new serogroup E of *Pasteurella multocida* was identified.

### Bluetongue disease

Pentavalent bluetongue inactivated vaccine using montanide and saponin adjuvant was developed by two centres separately using Indian strain. The vaccine was released for commercialization. Eight isolates of BTV were isolated from goats and culicoides midges for the first time in India. Three new serotypes, i.e. BTV-3 (Kolkata and IVRI, Izatnagar) and BTV-16 and 21 (Hyderabad) were confirmed by RT-PCR and nucleotide sequencing. Thus 39 BTV isolates of 9 different types were deposited and maintained at the IVRI, Mukteswar.

### Vaccines and therapeutics

- Complete HN and F genes of velogenic Newcastle disease virus responsible for outer membrane proteins (hemagglutinin-neuraminidase and fusion) were cloned in mammalian expression vector pVIVO-II-mcs

for use as bi-cistronic DNA vaccine.

- Field trials of buffalo pox, orf, sheep pox, combined goat pox and PPR vaccines and safety testing of live attenuated camel pox vaccine revealed no adverse reactions.
- PLG nanoparticles encapsulating outer membrane proteins of *Salmonella Gallinarum* induced good IgA antibody response at local mucosal sites following oral immunization in chickens, for use as a suitable mucosal vaccine delivery system.
- The siRNA based antiviral therapeutic against rabies virus and bovine herpesvirus-1 was successfully analyzed in *in-vitro* cell culture.
- Staphylococcal lytic phages encoding anti-staphylococcal peptidoglycan hydrolyzing enzymes were cloned and expressed. Native peptides revealed a broad range of anti *Staphylococcus aureus* activity and are potential therapeutic candidates for mastitis therapy.

### Diagnostics

Diagnostics like Taqman based one-step real-time RT-PCR, direct-ELISA and LipL32 for pestiviruses, brucellosis in sheep and goats and leptospirosis in cattle, respectively, were standardized.

### Molecular characterization of pathogens/receptors

- Sequencing of viral genomes from chicken, duck, goose and crow from west Bengal, Assam, Tripura and Sikkim revealed two phylogenetic groups of H5N1 viruses circulated during the period between January 2008 and May 2009, and the major group is closely related to isolates from Bangladesh.
- Out of 3 isoforms of  $\beta$ -tubulin gene,  $\beta$ -tubulin gene 2 could be identified as candidate gene for molecular detection of benzimidazole resistance of *Echinococcus granulosus*.

### Ethno-veterinary medicine

Methanolic extract of *Ocimum sanctum* and *Tinospora cordifolia* showed potential against canine demodicosis. *Aegle marmelos (bel)* was effective in restoring ovarian cyclicity and improved fertility in anestrus buffaloes.

### Surgical and clinical interventions

- A cervical splint for stabilization of fractures and dislocations of cervical vertebrae in dogs was successfully fabricated.
- The EDC-48 grafts were suitable for repair of skin and abdominal wall defects in rabbits.
- A cart for rehabilitation of dogs suffering from posterior paresis was developed.

## SUCCESS STORY

### Characterization of equine influenza virus isolated from 2008 outbreak in India

The disease was first diagnosed in Katra (Jammu and Kashmir) in last week of June 2008 wherein approximately 15,000 ponies and mules exhibited clinical signs. Subsequently, from July 2008 to March 2009, the disease was reported from Jammu and Kashmir, Himachal Pradesh, Delhi, Uttar Pradesh, Haryana, Rajasthan, Maharashtra, Karnataka and West Bengal. Equines (2,414) from these states were screened, and 438 samples (18.14%) were found positive for EIV antibodies.

Typing of EIV isolates (5 from J&K and 2 from Karnataka) indicated H3 subtype isolates. Sequence analysis of haemagglutinin gene of A/eq/Katra/06/08(H3N8) showed more than 98% sequence homology with A/eq/New Market/5/03, A/eq/Bari/05 and A/eq/Kentucky/5/02 isolates. Phylogenetic analysis of the HA gene confirmed the virus to be of Clade 2 of the Florida sublineage within American lineage. The HA1 gene sequence matched most closely to the isolates from China and that of Mongolia indicating introduction of the virus in India from northern international borders.

### Quality control and production of veterinary biologicals

Different doses of viral vaccines (> 7 lakh), bacterial vaccines (2,870 doses) and diagnostics (91,194 ml) in addition to monovalent FMD vaccine (19.60 million) were produced and supplied to various establishments.

### Equine diseases

A type-specific ELISA using EHV-1/4 recombinant glycoprotein G was developed. Multiplex PCR was further validated with 29 clinical samples suspected of EHV1/4, which differentiated the pathogens as EHV-4. Immuno-stick ELISA was developed as a pen-side test substitute to plate-ELISA for field diagnosis.

A laboratory-oriented kit was developed for equine piroplasmiasis. The validation of this kit using OIE-approved CI-ELISA kit revealed DSP and DS<sub>n</sub> values of 0.97 and 0.96 for NRCE kit, which were comparable to DSP (0.95) and DS<sub>n</sub> (0.93) of OIE-approved CI-ELISA kit.

### Veterinary type culture

At the recently established Veterinary Type Culture Centre standard operating procedures (SOPs) for animal cell culture, bacterial culture, and preservation were standardized.

### Field trial of equine herpes virus-1 vaccine:

A field trial of an inactivated EHV-1 vaccine for control of abortions in pregnant mares was

conducted, and its efficiency was checked with a commercial vaccine. Two booster vaccines were given at seventh and ninth month of gestation. Following vaccination, no adverse effect was observed. Response of vaccine was comparable to that generated by the commercial vaccine. This study revealed that vaccine developed by the NRCE could afford protective immune response in vaccinated animals and does not produce any untoward reaction in pregnant mares.

### Diseases trend

**Buffalo:** Retrospective epidemiological analysis revealed that maximum mortality (7.03%) was in neonatal calves (< 1 month of age). Mortality trends revealed 29.17% pneumoenteritis, 27.15% pneumonia and 24.75% enteritis. Suitable package of practices have been developed for addressing the problem at field level.

**Yaks:** Yaks were screened by CHEKIT-*Chlamydomphila abortus* enzyme linked immunosorbent assay for the detection of *Chlamydomphila abortus* specific antibodies. The overall prevalence in yaks was 34.88%. The prevalence of *Chlamydomphila abortus* specific antibodies was significantly higher in yak cows (40.74%) than among the bulls (25%).

*Escherichia coli* (EPEC) isolates from captive yaks revealed that 28.76% yak possessed at least one virulence gene. Among them 26.02% STEC and 2.28% EPEC strains were isolated. PCR showed that 35 (55.55%) isolates carried *stx1* gene, 51 (80.9%) possessed *stx2* gene and 10 both *stx1* and *stx2* gene.

**Poultry:** Administration of ND vaccine through feed and water was compared with that of regular intraocular vaccination in Gramapriya female line at PDP. HI antibody titers were 7.3, 6.5 and 7.5, respectively, in water, feed and ocular methods, respectively. Acidified iodine, peroxygen compound and acetic acid at 1%, 0.5% and 0.5% concentration, respectively, in hard water and soft water completely inactivated NDV both in the presence and absence of organic matter with 10 minutes of contact time.

## FISHERIES

### Capture Fisheries

#### Marine fish landings and catch structure:

The marine fish landings in India during 2008–09 touched the 3.21 million tonnes mark with an increase of about 0.327 million tonnes (11.3%) against the estimates of the previous year. Pelagic finfishes constituted 53%, demersal fishes 27%, crustaceans 15% and molluscs 5% of the total landings. The west coast was the highest contributor

among regions and among states, Kerala (21%) followed by Gujarat (19%). Among the different commercially important varieties, oil sardine (*Sardinella longiceps*) was the single largest contributor to marine fish landings with 444,593 tonnes (14%) during the year, followed by penaeid shrimps (6.4%).

The estimated value of the total marine fish landings at the primary market level increased by 29% over 2006 touching Rs 171,330 million and earning over Rs 249,340 million in the retail market level. The percentage share of fishermen in consumer rupee (PSFCR) has also increased over the years.

High value fish like coastal tuna and oceanic tuna registered growth levels of 23% and 39% respectively. Studies on the exploitation pattern of oceanic tuna indicate its potential as a possible candidate for further exploitation while coastal tuna production has nearly reached optimum level.

The targeted fishery for the deep sea sharks on the west coast landed more than 14 species of sharks as well as chimaeras. Bramble shark landings by drift gill netters (DGN) is on the increase in Cochin during post trawl ban period.

## Culture Fisheries

### Costal aquaculture

**Feed for mud crab:** Digestibility coefficient for dry matter, protein and energy of sardine fish meal, *Acetes* meal, soya flour, wheat and rice was determined to ensure cost effectiveness in the formulation of an artificial feed for mud crab. Apparent energy digestibility was highest in fish meal and *acetes* meal (about 95%) in animal ingredients. Soybean meal also had high apparent digestibility (92%). The digestibility of rice and wheat was lower than that of the animal ingredients. The dietary requirement of calcium and phosphorus for mud crab *Scylla tranquebarica* was also

### Myctophids (lantern fishes) – A potential deep sea resource

Deep sea shrimp trawlers operating off Kerala coast are harvesting huge quantities of *Myctophids* as by-catch. Large concentrations were observed in offshore areas at a depth range of 350–450 m. On an average, in a single day time operation, 250 kg of myctophids were caught off Quilon coast, which form about 41.6% of the total catch. This fishery has a good potential in fish meal industry and in formulation of value added products. The bycatch included three species of genus *Diaphus* (*Diaphus watasei*, *D. thiollieri* and *D. garmani*) and two species of genus *Myctophum* (*M. obtusirostre* and *M. fissunovi*). *Diaphus garmani* was recorded for the first time from Indian waters.

### Utilization of salt affected lands for aquaculture—A successful carp seed rearing operation

Under the NAIP sub-project “A value chain on fish production in fragile agriculture and unutilized aquatic resources in Maharashtra”, utilization of salt affected lands in western Maharashtra was taken up for carp seed rearing operations. The CIFE, Mumbai, developed demonstration units in the society-owned lands at the villages of Shere, Gondi and Malkhed, (Karad, Satara district, Maharashtra). Under the scheme, 0.25 million carp seeds were successfully raised and marketed. About 0.2 million fingerlings were raised to yearlings. Three ponds of 0.4 ha were stocked with carp fingerlings which were grown up to 250–300 g in 3 months. These results clearly showed the potential of utilization of salt-affected lands for aquaculture.



Fish Biomass Sampling (Karad)

determined using juvenile mud crabs. Diets containing 1–4% calcium showed better weight gain and minimal inter-moult duration (61 days to 56 days). Crabs fed with a diet without phosphorus showed poor weight gain and increase in carapace width. Diets containing 1.0–2.5% phosphorus showed better weight gain and increase in carapace width with minimal inter moult period (66 days to 52 days).

**Biosecured zero water exchange system:** The biosecured zero water exchange system technology adopted by farmers was further refined to improve the biosecurity protocols in shrimp farming. Use of beneficial microorganisms (*Lactobacillus*, *Bacillus*, *Pseudomonas* and probiotic yeast *Saccharomyces*) for controlling the pathogenic microorganisms and maintaining water quality was one of the major interventions in the refined technology. Under on-farm trial, an average production of 3,109 kg/ha, 80% survival, 7.95% gain in terms of production, 6.96% gain in survival rate and 10.24% gain in FCR were obtained in experimental ponds in comparison to the control ponds. The technology is ready for on-farm demonstration and dissemination to farmers.

**Microfeeds for nursery rearing:** A microdiet, with a nutrient profile of 45% protein and 12% lipid, was developed for the nursery rearing of

### Demonstration of carp farming system in NEH Region

The new "Composite Carp Farming" technology involving three exotic carps namely grass carp, silver carp and common carp stocked @ 3–4 fish/m<sup>2</sup> in a combination of 40–45%, 20–25%, 35–40%, respectively along with 10% rohu or chocolate mahseer has been demonstrated in farmers' pond in Manipur and Arunachal Pradesh. Supplementary feed was provided to the stock @ 2–3% of the body weight on daily basis and pond fertilization was done with organic fertilizers @ 9,000 kg/ha. The technology has been successfully adopted by the fish farmers in five districts of Manipur (21 farmers) and 13 districts of Arunachal Pradesh. Local KVKs and one NGO are also helping farmers in the dissemination of carp culture technologies.



Fish farm at Zero, Arunachal Pradesh

seabass fry (*Lates calcarifer*) and was successfully tested in a farm. The performance of the nursery feed was good with a survival rate of 50–72% in 30 days and the fishes attained an average body weight of 2–4 g. This feed technology is ready for commercial scale production.

### Screening for emerging shrimp diseases:

Emerging diseases, loose shell syndrome (LSS) and monodon slow growth syndrome (MSGs), besides white spot disease are affecting shrimp farming. Screening of the causative factors of LSS in black tiger shrimp farming confirmed the involvement of a filterable viral-like agent of about 13–24 nm size. This was purified from LSS affected shrimp. Transmission electron microscopic examination of the purified putative agent showed enveloped oval to elliptical shaped structures with a central dense core. Similar agents were also observed in nucleus of hepatopancreatocytes of LSS affected shrimp. LSS could be induced in healthy tiger shrimp by experimental challenge using purified virus-like agent and homogenates of hepatopancreas and muscle of LSS affected shrimp. Secondary bacterial infections caused by *Vibrio* sp. were common among these shrimps. Commercial products/compounds indicated that tested products could not mitigate the disease either in terms of prophylactic or control measures.

Farm grown *P. monodon* from farms with a history of extended culture period and growth retardation suspected to be affected by monodon slow growth syndrome (MSGs) were screened for Laem-Singh Virus (LSNV). Of the 110 samples collected, 83 (75.5%) were positive for LSNV. Screening of samples for co-infection by PCR showed positive results for IHNV (26%), WSSV (13%) and PvNV (3%). Though LSNV was reported from India based on sample from one location, this is the first report based on an extensive survey and future research will focus on the development of improved diagnostics for detection of LSNV based on metagenomics approach.

□