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For any editorial enquiry please contact:

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E-mail: cee@kab.delhi.nic.in

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Epidemiology of emerging viral zoonoses

G V ASOKAN[1], A GEORGE VASANTHAN A[2], V PRABHAKARAN[3] and S KOKILA PRABHAKARAN[4]

Institute of Veterinary Preventive Medicine, Ranipet, Tamil Nadu 632 402 India

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ABSTRACT

The last 2 decades has witnessed, peculiar disease emergence, which were sudden and hitherto unknown. New viral zoonoses are posing a greater challenge to public health. Most new zoonoses are due to infection with haemmorhagic viruses. Risk assessment and risk management are partial. The objective of this review is to create an understanding at the systems level, looking at the ecosystems, evolutionary biology, epidemiology and population dynamics of parasites, vectors and hosts. This retrospective information forms a strong basis for the theory of causation, thereby initiating the development of newer tools for diagnosis and working out the strategies for prevention.

Key words: Emerging virus, Epidemiology, Viral zoonoses

Emerging infections have been defined as diseases whose incidence in animals or humans have increased within the last 2 decades or threatens to increase in the near future (Lederberg *et al.* 1992). Studies of emerging infections typically rely on disease, organisimic, or syndromic approaches. Many disciplines relevant to research emergence lie outside traditional infectious disease training and research, and include evolutionary biology, demography, population dynamics, veterinary medicine, ecology, vector biology, climatology, epidemiology, genetics and behavioural sciences. The study of infectious diseases in other species may provide important insights into understanding the process of disease emergence in humans. The study is also relevant to understanding the species to species spread of organism (Mary 1999). The joint WHO/FAO Expert Committee (1967) on zoonoses have defined as —Those diseases and infections which are naturally transmitted between vertebrate animals and man. There are more than 400 infectious diseases and among them little above 150 are attributed to be zoonotic. It may be due to viruses, bacteria, rickettsia, fungi, helminths, protozoa, arthropods or insects (Steela 1979) and recently prions. In the last 2 to 3 decades, a significant increase of viral zoonotic infections was observed. These zoonoses are not only newly emerging, but also due to the reappearance of diseases thought to have been defeated. The suddenness and severity of a zoonotic outbreak may predict how much attention it will receive but not its eventual importance to the public health. New viral diseases can arise when viruses broaden host range (monkey poxvirus; equine morbilli virus) or the intrinsic properties of the virus itself such as high mutation rates (influenza A). Many of them are transmitted by insects (arbo viruses, eg: yellow fever) or by rodents (hanta viruses) others by contact with patients and nosocomial infections (ebola). Once introduced into human, zoonotic viruses that are not particularly pathogenic in their host species have produced noteworthy outbreaks of disease eg: marburg filo virus from vervet monkeys in Germany (1967) resulted in 2 generation of human to human infection with case fatality rate of 23%. A zoonotic Bunya virus in a Pakistani shepherd (1976) caused crimean congo hemmorrhagic fever and continued for 2 generation of human to human transmission. Similarly, the zoonotic filo virus ebola resulted in large nosocomial outbreaks in Sudan (1976) and in Zaire (1976, 1979, 1995) with case fatality rate of 80 -- 90% with multiple generation of human to human transmission (Louis *et al.* 1995, Greiser *et al.* 1999).

The factors that contribute to the emergence of new zoonotic diseases are:

(i) Global human and livestock population's continued growth bringing them close to each other.
(ii) Advancement in transportation (circum navigation of the globe in less than the incubation period of infectious agents)
(iii) Microbial determinants such as mutation, natural selection and evolutionary progression
(iv) Individual host determinants such as acquired immunity and physiologic factors
(v) Host population determinants such as host behavioural

Present address: [1,3,4]Institute of Veterinary Preventive Medicine, Ranipet 632 402.
[2]Department of Biotechnology, Vellore Institute of Technology, Vellore 632 006.

characteristics and societal,transport, commercial and iatrogenic factor
(vi) Environmental determinants such as ecologic and climatic influences
(vii) Bioterroristic activities

The evidence for zoonotic transmission is substantiated by

(i) Similarities in viral genome organisation
(ii) Phylogenetic relatedness
(iii) Prevalence in natural host
(iv) Geographic coincidence
(v) Plausible routes of transmission (Frederick 1998)

Australian bat lyssa virus

Lyssa virus is closely related to classic rabies virus and was identified in 1996 in Australia, in a fixed brain specimen from a young black flying fox, with unusual neurologic symptoms. Since this original isolation, a further 42 isolates have been identified from fruit bat species. Field *et al.* (1999) analysed using nucleocapsid – specific monoclonal antibodies and showed a strong relationship between this new lyssa virus and sero type I rabies virus. It is referred to as ballina virus in US. The prevalence among bats is 3.4%. Two human deaths have been attributed to lyssa virus. The death of the second victim happened 2 years after a bite. The lyssa virus expert committee suggests that rabies vaccine and immunoglobulin may elicit a protective immune response in humans against lyssa virus. The finding of well, antibody positive bats, suggested that bats can either recover from infection or that they can be silently infected, needs to be investigated, particularly with respect to infectivity, possible transmissibility and virus ecology (Gould *et al.* 1998).

Borna disease virus (BDV)

It causes a rare neurological disease in horses and sheep. The virus has not been classified because neither an infectious particle nor a specific nucleic acid has been identified. The clinical course depends on animal species, age, virus strain and route of infection. This slow virus causes excitability, apathy, spasms and partial paralysis and the disease is usually fatal. BDV is enzootic in Germany and Switzerland for over 150 years (Narayan *et al.* 1983, Hirokazer *et al.* 1997).But it was Bode *et al.* (1996) who first isolated the agent from patients with mood disorders and gave a direction on its zoonotic importance. Among the normal population 2% prevalence has been recorded and higher prevalence of 13 – 14% were present among patients with chronic progressive diseases of the brain and immune system. Among the HIV infected individual the proportion of BDV antibodies were 4- times higher (Bode *et al.* 1992).

Hanta virus pulmonary syndrom

Hanta viruses, a rodent-borne RNA virus, is found worldwide. The old world hanta viruses, such as Hantaan, Seoul, and Puumala, long known to be associated with human disease, cause haemorrhagic fever with renal syndrome of varying degrees of severity (Schmaljohn and Hjelle 1997). The 1993 outbreak of hanta virus pulmonary syndrome (HPS) in the 4 corners region of south western United States was associated with Sin Nombre (SN) virus, a rodent- borne hanta virus; the virus's primary reservoir is the deer mouse. The population of this rodent vector expanded following a dramatic increase in precipitation associated with El Nino phenomenon, which resulted in abundance of rodent food sources (vegetation and insects). There was a 20-fold increase in the rodent population compared to the previous year and the likelihood of more rodent – to- rodent contact, rodent –to-human contact, and viral transmission, there by resulting in large number of cases in 1993 and 1994. Following an environmental stress after El Nino rodents competed for food and moved in to homes and peridomestic structures increasing the rodent –to- human contact. The virus is transmitted to humans through inhalation of aerosolized faeces, urine or saliva from infected rodents. Rodent densities decreased to normal levels in 1995. Three viruses other than SN have been associated with HPS in North America. All cases associated with viruses other than SN have occurred outside the range of the deer mouse. The viruses are New York virus, Black creek canal virus and Bayou virus (Mckee *et al.* 1991, Paul *et al.* 1999).The infection caused by this virus was previously unrecognised because of its low prevalence and the lack of available techniques to identify the agent. The syndrome was recognised after a sudden cluster of human cases suggested a hanta virus disease on the basis of clinical and epidemiological characteristics. An incubation of 2 to 3 weeks is typically followed by high fever, chills, myalgia, fatigue, and gastrointestinal manifestations. This phase is followed by abrupt onset of dysponea and hypoxia, typically associated with noncardiac pulmonary edema, and respiratory failure. Renal dysfunction and shock are also observed. However, 5 cases were reported without pulmonary involvement suggesting that HPS surveillance has to be expanded (Nichol *et al.* 1993, Duchin *et al.* 1994, Engelthalen *et al.* 1998).

Hendra virus

Hendra virus was only found in Queensland where 2 men and 16 horses died. Formerly known as equine morbilli virus was first isolated in 1994, after an explosive outbreak in Hendra, a Brisbane suburb (News 1999). A second incident occurred in Mackay, 1 000 km from Brisbane. Two horses and a farmer died, the latter from severe meningoenchephalitis. No connection was found between the 2 incidents. Fruit bats (flying foxes) are the natural hosts on serologic grounds and by virus isolation. Serologic evidence of infection of fruit bats has also been reported from Papua New Guinea. There is no evidence of seroconversion among bat care givers, despite their close contact with bats. Furthermore, all human infections appear to have been transmitted from horses Thus

the virus appears to have low transmissibility to humans; and appears to be linked with pregnancy. In both the incidences in Australia, pregnant mares were involved, infection occurred during the birthing season of flying foxes, and virus was first recovered from uterine fluid of a pregnant animal. Much has to be answered about the ecologic, biologic and pathologic characteristics of hendra virus. The infectivity, virulence, transmissibility, role of pregnancy, latency and the tropism of the virus are the future research areas The Nipah virus is related to but distinct from the Hendra virus in term of the range of species they infect and the way in which the 2 viruses appear to be transmitted. There is about 10% difference in the amino acid sequence between the 2 viruses (Murray *et al.* 1995, Rogers *et al.* 1996,O'Sullivan *et al.* 1997).

Influenza virus

The influenza type A (H5NI) virus was first detected when outbreaks on poultry farm occurred in Hong Kong (1997). The strain represented a viral shift-a major change in viral surface antigens from the predominant Wuhan (H3N2) strain. All the isolates from humans contained anything but avian genes which suggest that each case has been acquired from poultry. Professor Kennedy Shortridge, of Hong Kong University concluded that human cells have fewer receptors for avian influenza virus genes. Sporadic H5NI infections may occur in human, but the virus is unlikely to spread efficiently among human unless receptor significantly changes. The current understanding is that pandemic strains arise when a prevailing human influenza virus and an avian influenza virus co – infect an intermediate host, probably, the pig, which has receptors for the surface haemagglutinins of the 2 viruses. Reassortmant of the 2 viruses in the intermediate host could then give rise to a new strain that can infect human. Between March to mid May 1997, H5NI resulted in death of more than 95% of birds on 3 farms in close proximity. Approximately 1.5 million birds were slaughtered and 5 people died out of 17 clinical cases in Hong Kong (Editorial 1997,Sims 1997).

Nipah virus

Outbreak of an encephalitic illness among pig farm workers and abattoir workers in Malaysia and Singapore were reported in 1997. In 1999, 258 cases of encephalitis were recorded in adults with a case fatality rate of almost 40%. The cause turned out to be a hitherto unknown new mega myxo virus within the family of paramyxo virus. The virus was named nipah, a village affected in the Malaysian state of Negri sembilan. The virus was responsible, for death of thousands of pigs, and a small number of other animals including horses, cats, dogs, fruit bats (flying foxes) and goats. The first nipah virus cases occurred at a farm where fruit trees (frequented by fruit bats) were planted within metres of pig pen. It is believed that the outbreak may have originated when infected fruit bats came into contact with the farmed pigs. In Malaysia over 900 000 pigs from affected areas were culled (Jeremy 1999)

The virus caused mild to severe signs including fever, headaches, occasionally drowsiness and disorientation sometimes resulting in coma. The majority of the patients in a coma subsequently died. The suggestion that virus multiplied in tonsils, respiratory epithelium and contaminated cellular debris of the air passages in the lungs raises the possibility of its transmission by pharyngeal and bronchial secretions. Person to person transmission was unusual and mosquitoes or other insect vectors did not transmit the disease. To date, cases of infection with nipah virus have been reported only in Malaysia and Singapore. However, infected animals including bats could spread the disease further (Nicholas *et al.* 1999, Kaw *et al.* 1999).

Retro viruses .

The primate T- lymphotrophic virus I spread during its evolution through repeated interspecies transmissions between primates and is now present in many species of old world monkeys, apes and in humans (Gouban *et al.* 1996). Several strains of human immunodeficiency virus-2 (HIV) in west Africa were independently derived from simian immuno deficiency viruses (SIV), the virus that is endemic in sooty mangabey monkeys populating that region. Feng *et al.* (1998) provided the most persuasive evidence that HIV–1 came to human from chimpanzee, *Pan troglodytes troglodytes* which harbours the related SIVcpz. The human T-lymphotrophic virus-I (HTLV) has also originated more than once from related simian viruses including simian-lymphotrophic virus-I (STLV) of chimpanzees. The human pygmies in Gabbon are infected with a strain of HTLV – I, that is virtually indistinguishable from STLV – I of mandrills living in the same forest.

West Nile like viral encephalitis

An arboviral encephalitis outbreak was first recognised in last August 1999. West nile like viral encephalitis (WNV) has never been isolated in the western hemisphere. Although initially attributed to St. Louis encephalitis, (SLE) later it has been confirmed as a west nile like virus (WNV), based on the identification of virus in human avian and mosquito species. It is a flavi virus belonging taxonomically to the Japanese encephalitis sub group that included the serologically closely related (SLE) virus, Kunjin virus, Murray valley encephalitis virus and others. Probably migratory birds or imported birds or international travel of infected persons would have transmitted it. It is still unclear whether the virus that caused this outbreak in previously identified strain of WNV or a new variant. A total of 17 confirmed cases, 20 probable cases and 4 deaths were recorded from New York and the surrounding counties (Asmis *et al.* 1999).

Xenozoonoses

Xenoses and xenozoonoses are infectious illnesses introduced into humans through procedures involving xenogenic tissue. The use of xenogenic tissues has to be

examined in the light of the experience with viral zoonotic infections particularly with recognized zoonotic pathogens and unknown xenogenic agents (hanta virus, cercopithecine herpes virus 1 – B causes encephalitis). The endogenous viruses are xenotrophic and cause even more uncertainty than the exogenous retro viruses (Louis *et al.* 1995).

Other zoonotic viruses

Mayaro virus disease (MVD) is an emerging mosquito-borne viral illness in tropical South America. Twenty-seven cases have been recorded. It is a nonfatal dengue like illness characterized by fever, chills, headache, generalized myalgia, diarrhoea, vomiting and rash of 2 – 3 days duration. Severe joint pain is the feature of the illness. MVD and dengue are difficult to differentiate clinically (Tesh *et al.* 1999). Puumala virus from bank voles causes nephropathia epidermica. Herpes virus simiae or B-virus is an emerging threat in the United States since 1990,particularly among macaque pet owners and laboratory workers handling infected brain and kidney tissues. Incubation period may be as short as 2 days, but more commonly is 2 to 5 weeks. Viral infection rapidly progresses to central loci in the spinal cord and, eventually, the brain. Of 24 known symptomatic patients whose cases were reviewed in 1992, 19 (79%) died (Stephanie *et al.* 1998). A new paramyxo virus, menangle virus, was identified from cases of abortion in pigs and an influenza like illness in workers at a single piggery in New South Wales, Australia, and in a university teacher. Field studies confirmed that bats were likely to be the primary hosts of both the hendra and menangle virus (Gardner 1999). Cache valley virus caused fatal encephalitis in humans. The virus, a mosquito- borne member of the bunyanwera sero groups which has morphological characteristics of the family bunyaviridae, of arbo virus had previously been isolated only from vertebrates and mosquitos but never from a human. It has been reported (Balasubramaniam 1999), recently, that Hepatitis C virus and bovine viral diarrhoea virus are remarkably similar.

The most recent emerging calici virus is associated with rabbit haemorrhagic disease virus (RHD), and although an association has not been reported, the agent readily moves between continents crosses ocean channels. The RHD virus is closely related to other hepatotrophic calici virus, hepatitis E. RHD virus causes fever, gastroenteritis, neurologic symptoms, skin rashes, bleeding and hepatitis (Barlough *et al.* 1986, Chasey 1994).

Steps in identification of suspected new viral agent

The tools used to study and understand disease emergence include mathematical modeling, geographic information system, remote sensing, molecular methods to study the genetic relatedness of organisms, and molecular phylogeny, paleobiology, paleoecology and studies that allow the reconstruction of past events may help inform future research and policy:

(i) Cell culture to cytopathic effect production. If culture negative, electron microscopy and consensus primer polymerase chain reaction (PCR) approach is followed.

(ii) In the absence of cytopathology, electron microscopy is used to know the physical parameters of the particle. If morphology is not seen in electron microscopy cDNA library screening is done.

(iii) A serologic approach could be taken and reacted against panels of viral antigens in an effort to uncover a serologic relationship between the antibodies in the patient serum and known viral antigens. When this fails, expression library screening, representational difference analysis (RDA) or PCR using consensus primers.

(iv). Collaborative surveillance and research (Richard 1999).

The zoonoses from endangered animals are more difficult to control than from domestic animals. There is no way to predict when (or) where the next important new zoonotic pathogen will emerge (or) what its ultimate importance might be. The disease control is at risk because of failed investigative infrastructure or financial base.

Investigation at the first sight of emergency and developing a coherent framework to advance the understanding of the process of disease emergence is important. Further, identifying the conditions, combinations and sequences of events that contributed to the changed pattern of infections is crucial. Such investigation will be a discovery to control continuum.

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Anthelmintic activity of *Xanthium strumarium* against *Haemonchus contortus* infection in sheep

S R SHARMA[1], D SINGH[2], F A KHAN[3], C P SWARANKAR[4] and P S K BHAGWAN[5]

Central Sheep and Wool Research Institute, Avikanagar, Rajasthan 304 501 India

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ABSTRACT

Anthelmintic activity of the fresh juice of *Xanthium strumarium* leaves at different concentrations was evaluated *in vitro*, against benzimidazole resistant *Haemonchus contortus* strain, using egg hatch assay (EHA) and larval paralysis test (LPT). No anthelmintic activity of the *Xanthium strumarium* juice was observed at 1: 100 dilution, however, undiluted juice of the leaves showed a 100% inhibition in egg hatching by EHA. The inhibition in egg hatching was 100% at 1: 2 and 1: 10 dilutions of the juice, whereas at 1: 50 dilution, it was only 21.5%, after 48 hr of incubation. The control mortality was 12.1%. In LPT, the percentage paralysis of the larvae at concentrations – undiluted, 1: 2, 1: 5, 1: 10 and 1: 100 were 23.8%, 25.5%, 21.5%, 17.7% and 5.44%, respectively, indicating significant ($P<0.05$) anthelmintic activity up to 1: 10 dilution of the juice. The per cent survivability of the larvae in the control group was 97.5.

Key words: *Haemonchus contortus*, Larvicidal effect, Ovicidal effect, Sheep, *Xanthium strumarium*

In an attempt to maximize the livestock production losses incidental to endo-parasitism, a frequent, indiscriminate and non-judicious use of anthelmintics against endoparasites had resulted in resistant strain (s) of the parasite (s). *Haemonchus contortus* (*H. contortus*), a common trichostrongylid worm parasite of sheep, goat and cattle has been no more an exception to this. The parasite has been documented resistant against drugs in all of the 3 broad spectrum families, viz. the benzimidazoles, avermectins–ivermectin, and imidazothiazole (Lacey 1988, Prichard 1990 and Prichard *et al.* 1980); and against drugs with narrow spectrum of activity such as salicylanilides (Rolfe *et al.* 1990). To sustain the livestock production, circumstances thus necessitates to develop new drug (s) / lead compound to combat the problem of endo-parasitism.

Xanthium strumarium (*X. strumarium*) L. (family-compositae) [Hindi name – *Chhotta Gokharu*] a gregarious weed distributed throughout Indian tropics has been indicated as diaphoretic, sedative, sudorific, diuretic, sialagogue, anti-malarial (Nadkarni 1954), anti-cancerous (Mukherji *et al.* 1970), and is used by patients suffering from small pox (Chopra *et al.* 1958). It is also reported to possess hypoglycaemic (Dhar *et al.* 1968), antimicrobial (Jawad *et al.* 1988) and antitrypanosomal (Talakal *et al.* 1995) activities as well. This preliminary study deals with *in vitro* activities of the fresh juice of *X. strumarium* leaves against ova and larvae of benzimidazole resistant *H. contortus* of sheep.

Present address: [1]Senior Scientist (Veterinary Medicine), Project Directorate on Poultry (PDP), Rajendranagar, Hyderabad 500 030, Andhra Pradesh.

[2]Senior Scientist, [3,4]Scientist (Senior Scale), [5]Principal Scientist, Division of Animal Health.

MATERIALS AND METHODS

In September, fresh green leaves of *X. strumarium* were collected, washed thoroughly and spread under fan. The leaves were crushed finely in to a paste using mortar and pestle and the fresh juice was obtained by straining through double layered muslin cloth. The juice was stored in an airtight sterilized glass vial at 4°C and was used within half-an-hour in different dilutions with triple distilled water.

Two donor lambs, infected with benzimidazole resistant strain of *H. contortus* were used for obtaining eggs and larvae for tests, viz. an egg hatch assay (EHA) and larval paralysis test (LPT). Parasitic eggs were recovered by standard method (Taylor 1990) and the final dilution was adjusted to 100 eggs per 10µl. The larvae were separated by the Baermann technique (Hansen and Perry 1990) from the coproculture (s) and their concentration was adjusted to 200 larvae / ml of distilled water.

In vitro EHA as described by Coles *et al.* (1992) was used with slight modification to assess the ovicidal efficacy of the fresh juice of *X. strumarium* leaves. The fresh juice of *X. strumarium* leaves was used as undiluted, and in 1: 2, 1: 10, 1: 50 and 1: 100 dilutions. To 1 ml of the fresh juice (undiluted or diluted) in multiwell plates, about 10 µl of freshly collected

egg suspension was added and 3 replicates were made for each drug concentration. In control well, distilled water was used in place of the juice. The plate (s) were incubated at 27°C in a large covered glass petri-dish sealed with parafilm. After 48 hr, all eggs / larvae from each well were examined under 40- times magnification and percent inhibition of hatching was calculated.

In vitro LPT as described by Martin and LeJambre (1979) was used with modification. The fresh juice of *X. strumarium* leaves was used as undiluted and in dilutions of 1: 2, 1: 5, 1: 10 and 1: 100, while control well containing distilled water was used. Test system comprise 1ml of test drug and 1ml of L-3 larvae suspension in a multiwell plate duly marked for identification of each concentration in triplicate. Larvae in suspension were examined after 24hr of incubation at 25°C, for their viability and motility at 40X magnification and were analyzed as per cent paralyzed / dead. All data so generated on EHA and LPT were subjected to ANOVA (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Data on effect of the *X. strumarium* leaves aqueous suspension on hatching of *H. contortus* eggs (EHA) is presented in Fig.1. The test drug exhibited a significant ($P<0.05$) ovicidal and inhibition of hatching activities in undiluted suspension and in 1: 2 and 1: 10 dilutions than activities at 1: 50 dilution onward and in control. The findings indicated that the test juice exhibit ovicidal and inhibition of hatching effects up to its 1: 50 dilution.

The effect of the preparation on the L-3 larvae in LPT is depicted in Fig. 2. The juice had significant ($P<0.05$) larvicidal

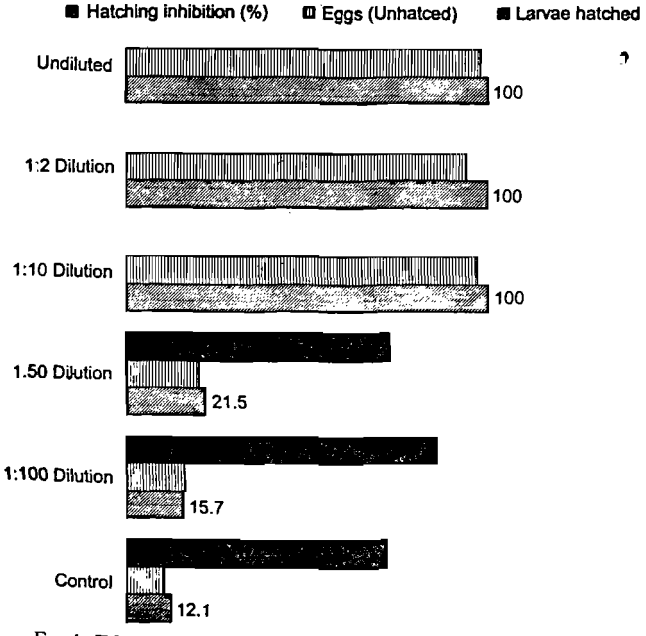

Fig 1 Efficacy of *Xanthium strumarium* leaves on *H. contortus* (egg hatch assay).

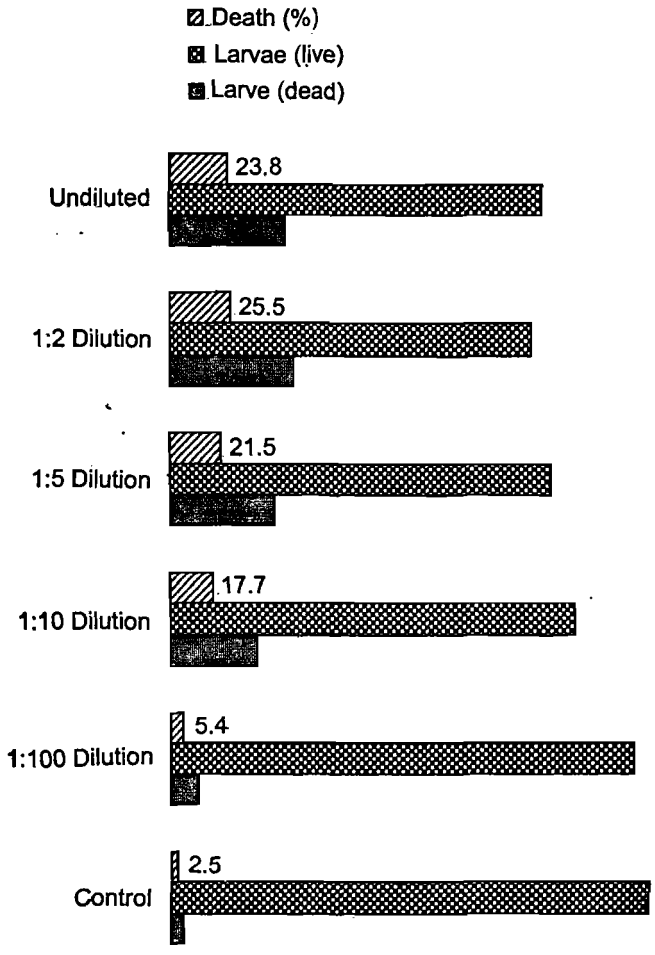

Fig. 2. Efficacy of *Xanthium strumarium* leaves on *H. contortus* (larval paralysis test).

effect at undiluted, 1: 2, 1: 5 and 1: 10 dilutions than to its 1: 100 dilution and control. Evidently, the survival of larvae increased as the dilution of the test drug increased. It was interesting to note that the test juice had almost similar larvicidal activity up to 1: 10 dilution under the present test system.

Obviously, the fresh *X. strumarium* leaves juice had significant ovicidal and larvicidal activities, *in vitro*, against the benzimidazole resistant *H. contortus*. Chemically, the leaves of *X. strumarium* are known to contain alkaloids and sesqueterpene lactones like xanthinin, xanthumin and xanthatin (Minato and Horibe 1965, Winters *et al.* 1969); isohexacosane, chlorobutanol, stearyl alcohol, ß-sitosterol, α-sitosterol, palmitic acid (Bisht and Singh 1977); xanthanolides, viz. 2-epixanthumin and 8-epixanthalin 5 ß-epoxide (Bohlmann *et al.* 1982); a pentacyclic lactone (Ahmad *et al.*1990) and xanthanolides (Malik *et al.* 1993, Saxena and Mondal 1994). Its one of the principle, xanthinin, had been attributed to possess antimalarial effect (Badam *et al.* 1988)

besides possibly antitrypanosomal activity as well (Talakal *et al.* 1995). The possibility that the ovicidal and larvicidal effects, as observed herein, were due to xanthinin alone or a synergistic with other active principles of the *X. strumarium,* could not be ruled out. More studies are, therefore, needed on the fractionation and identification of compounds having activity against the nematode and non toxic to the sheep.

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Sequential pathological changes in broiler chickens following experimental *neem* (*Azadirachta indica*) seed-cake feeding

DEEPIKA LATHER[1], S K MISHRA[2], SANDEEP GERA[3] and MEENAKSHI VIRMANI[4]

CCS Haryana Agricultural University, Hisar, Haryana 125 004 India

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ABSTRACT

Neem seed-cake fed birds showed pale and anaemic muscles. The relative organ weights of spleen, bursa of Fabricius, liver and heart did not reveal any significant change. Gross changes observed included varying degree of congestion and haemorrhages in lungs, intestine, spleen, pancreas, proventriculus and kidneys. Thickening of wall and excess mucus covering mucosal epithelium of intestine and proventriculus was evident, heart revealed hydropericardium and fibrinous exudate, whereas necrotic foci on liver in a few chicks. Histopathological studies revealed mild perivascular and perineuronal oedema with degenerative changes in brain, mild cystic spaces, oedema and fibroblasts between bursal follicles. Catarrhal enteritis and proventriculitis were also evident. Lungs revealed congestion, haemorrhages and peribronchial lymphoid aggregates, whereas liver showed dilated sinusoids, degenerating hepatocytes and kupffer cell hyperplasia and few necrotic foci. Kidneys revealed congestion, haemorrhages and tubular degeneration. Heart revealed heterophillic infiltration and mild degenerative changes in myocardium along with fibrinous pericarditis in a few broiler chicks.

Keywords: Pathology, Broiler chickens, *Neem* seed-cake

Neem seed-cake (NSC) is a byproduct of oil extraction from *neem* seeds and a good source of crude protein (16-19%) with a favourable amino acid profile (Singhal and Mudgal 1983). Extracted seed materials, when used in feed mixture for various types of birds, have been found comparable to peanut as a feedstuff (Gupta *et al.* 1975). It is therefore necessary to evaluate *neem* seed-cake to establish adverse effect if any, and ascertain a safe dietary inclusion rate before using it commercially as a protein supplement in broiler ration. Perusal of the literature revealed a limited information in respect of pathological changes in the broiler chickens following *neem* seed-cake feeding, which may be of some help in understanding the pathology of NSC, if any. The present paper places on record the sequential pathological changes in the broiler chickens following feeding of *neem* seed-cake.

MATERIALS AND METHODS

Day-old broiler chicks (120) procured from a commercial hatchery were reared under strict hygienic conditions. At 4 weeks of age, the chicks were randomly divided into 4 groups i.e. 1, 2, 3 and 4 (comprising 30 birds each) and were given 0, 5, 10 and 15% *neem* seed-cake in the feed. Three chicks from each group were sacrificed at weekly intervals starting from fifth week till the completion of the experiment (12 weeks of age) and subjected to thorough postmortem examination, and observations were recorded. The organ weight of heart, liver, spleen and bursa of Fabricius were recorded for all the 3 birds using electronic balance and their relative organ weight was calculated. Tissue pieces from bursa of Fabricius, brain, liver, trachea, lung, thymus heart, kidneys, spleen, intestine, pancreas, oesophagus, gizzard and proventriculus were collected and fixed in 10% buffered formalin. The tissue sections were cut at 4 µm thickness and stained with haematoxylin and eosin (Luna 1968) using conventional procedures.

Present address: [1]Technical Officer, Indovax Pvt. Ltd., 17-A, Defence Colony, Hisar. [2]Associate Professor, Department of Veterinary Pathology; [3]Associate Professor, [4]Research Associate, Department of Veterinary Biochemistry, CCSHAU, Hisar.

RESULTS AND DISCUSSION

Organ weight

The mean relative weight per cent of spleen in chicks of groups 1, 2, 3 and 4 ranged from 0.07±0.01 to 0.20±0.11, 0.06±0.007 to 0.16±0.09, 0.05±0.003 to 0.12±0.02 and 0.06±0.007 to 0.15±0.02%, respectively, at different intervals. There was no significant difference between the values in treated group as compared to control group.

The mean relative weight per cent of bursa of Fabricius in group 2 (0.09±0.02 to 0.19±0.03), group 3 (0.07±0.02 to

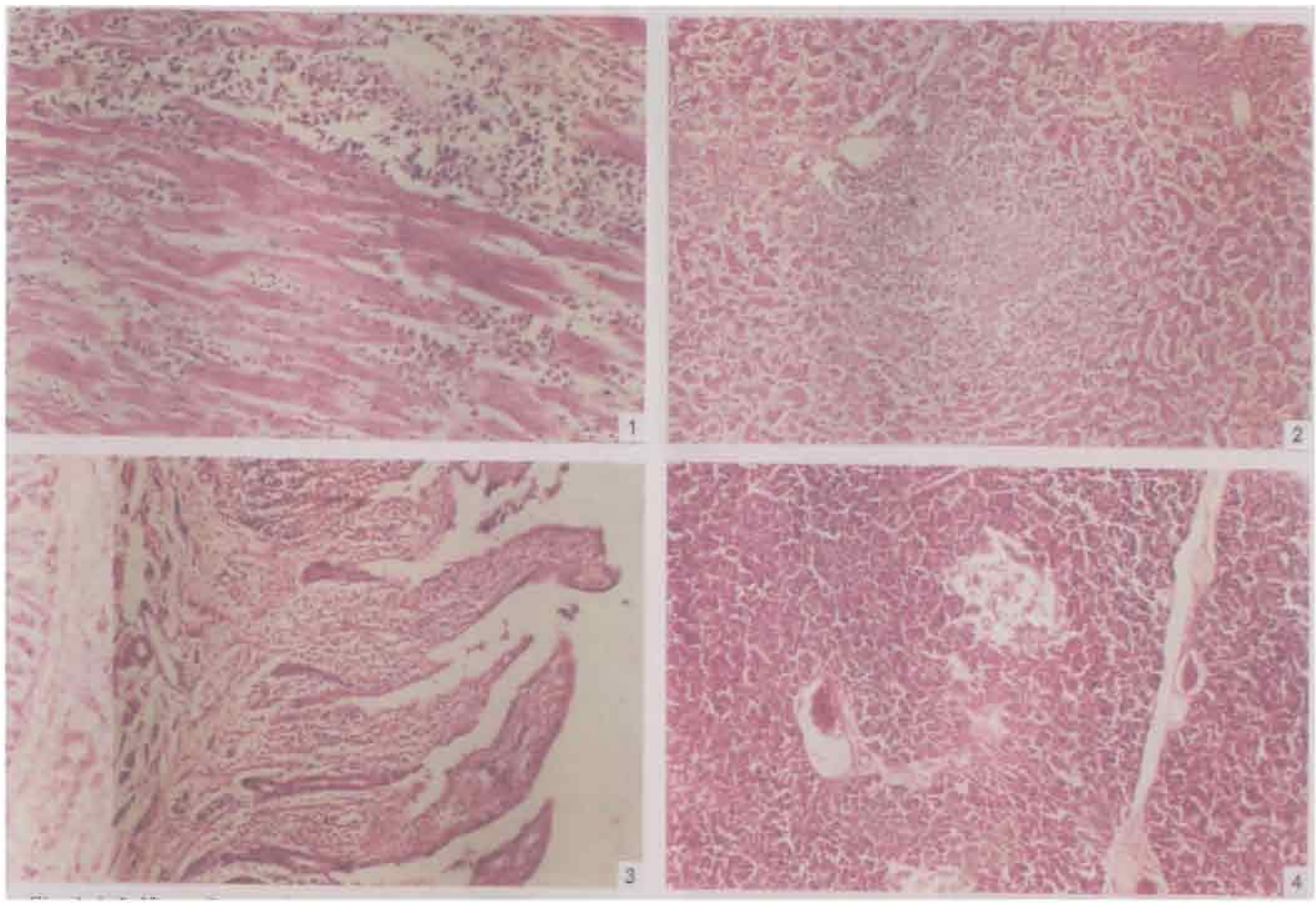
0.22±0.02) and group 4 (0.06±0.01 to 0.14±0.04) did not differ significantly as compared to corresponding control values (0.15±0.02 to 0.21±0.04).

The mean relative liver weight per cent ranged from 1.90±0.05 to 2.90±0.05 in control group, 1.91±0.30 to 3.56±0.60 in group 2, 1.76±0.15 to 3.28±0.86 in group 3 and 1.74±0.65 to 3.74±1.05 in group 4. There was no statistical difference in mean relative liver weights in NSC treated groups throughout the experiment.

The mean relative heart weight per cent ranged from 0.55±0.05 to 1.15±0.07 in control chicks, 0.58±0.03 to 1.31±0.40 in-group 2, 0.54±0.07 to 1.00±0.10 in group 3 and 0.59±0.06 to 0.89±0.17 in group 4. The values in groups 2, 3 and 4 did not differ significantly, ($P<0.05$) with control values at any interval.

Gross lesions

The general appearance of the carcass at necropsy was pale and anaemic which is in accordance with the findings of Christopher *et al.* (1976). It may be due to decreased haematological values e.g. Hb, PCV and TEC and reduced feed intake by experimental birds. There was congested intestinal mucosa and catarrhal exudate in the lumen of intestine and proventriculus. The lesions appeared more severe in groups 3 and 4 and continued till the end of experiment. In addition, haemorrhagic spots on duodenal mucosa were observed from 28 DPT onwards alongwith the thickening of the intestinal wall. The gall bladder appeared distended with dark coloured bile in all the NSC fed chicks till the end of the experiment. Mild congestion in liver, kidneys, lungs, pancreas and spleen was observed from 21 DPT till the 56 DPT, particularly in chicks of groups 3 and 4 and so also, necrotic foci on liver were present in a few chicks belonging to groups 3, 42 DPT onwards. Few chicks of groups 3 and 4 also revealed fibrinous pericarditis between 21 to 35 DPT. The changes observed may be due to the bitter triterpenoids present in the *neem* seed-cake. Some of the earlier workers have observed different lesions in different organs. Sadagopan *et al.* (1982) have reported haemorrhages on the pericardial sac in a few chicks upon feeding neem seed-meal at 20% level. While decrease in the size and presence of few haemorrhagic spots on spleen were observed by Christopher *et al.* (1976)



Figs 1-4. **1.** Heart. Group 3. 21 DPT. Heterophilic infiltration and degenerative changes in the myocardium. (H&E × 66). **2.** Liver. Group 4. 28 DPT. Necrotic foci in the hepatic parenchyma and perivascular reaction. (H&E × 33). **3.** Proventriculus. Group 3. 28 DPT. Desquamated mucosal epithelium with diffuse leucocytic infiltration and aggregates in the lamina propria. (H&E × 33). **4.** Pancreas. Group 4. 21 DPT. Congested vessels and degenerative changes in the Islets of Langerhans cells. (H&E × 33).

and Sadagopan *et al.* (1982). In accordance to the present findings, congestion in lungs has been reported by Ali (1987) and Hore *et al.* (1999) in goats and rats respectively.

Histopathological findings

There was mild congestion, perivascular and perineuronal oedema in cerebrum as well as in cerebellum. Mild degenerative changes in few of the neurons were also evident. The lesions were similar in all experimental groups and continued till the end of experiment i.e. 56 DPT but 42 DPT onwards, meningial congestion also appeared. Similar changes in brain have been reported by Hore *et al.* (1999) in rats and Ali (1987) in goats upon feeding *neem* leaves at 5% level.

Bursa of Fabricius revealed cystic degeneration and separation of bursal follicles in all NSC fed groups from 7 DPT till the end of experiment. In addition, few chicks showed appearance of fibroblasts below the bursal epithelium at 28 DPT. Mononuclear cells along with fibroblasts became evident between bursal follicles on 49 DPT that continued till the end.

Salient histopathological lesions in heart included congestion, mild degenerative changes and heterophillic infiltration in myocardium (Fig. 1). The cardiac lesions continued with the progression of experiment and became more severe particularly in groups 3 and 4. Serofibrinous exudate was present along with few leucocytes (heterophils and few lymphocytes) in the pericardium of a few group 4 chicks between 21 and 56 DPT. Microscopically, instestine revealed mild congestion and goblet cell hyperplasia in group 2 chicks, while in groups 3 and 4 chicks, there was desquamation of mucosal epithelial cells, leucocytic infiltration in lamina propria along with goblet cell hyperplasia. The lesions became severe with the progression of experiment. However, few chicks of group 4 showed severe congestion and haemorrhages extending up to the serosa on 35 DPT. The serosa appeared thickened due to serous fluid and leucocytes. Similar lesions in intestine have been reported by other workers (Christopher *et al.* 1976, Sadagopan *et al.* 1982, Chand 1987, Reddy and Rao 1988, Gowda *et al.* 2000) in poultry. These changes appear to be due to irritant action of the bitter limonoids present in raw *neem* seed-cake.

Kidneys revealed mostly the vascular and degenerative changes which were more pronounced in groups 3 and 4 chicks. Prominent lesions included congestion, hemorrhages, contracted glomeruli, vacuolation in tubular epithelium and eosinophillic masses in their lumen. On 21 DPT and onwards, pseudogiant cells appeared in the lumen of renal tubules particularly in groups 3 and 4 chicks.

Liver revealed mild congestion and degenerative changes in group 2 chicks, whereas in groups 3 and 4, there were fatty changes in hepatocytes, dilated sinusoids, Kupffer cell hyperplasia apart from vascular changes. There was increase in severity of hepatic lesions in group 3 and 4. In addition, necrotic foci along with leucocytic infiltration and perivascular reaction (Fig. 2) were evident in a few chicks at 28 DPT onwards. The degenerative changes in the liver and kidneys have earlier been reported (Sadagopan *et al.* 1982, Singh *et al.* 1985, Chand 1987) in poultry and also by Gowda *et al.* (1996) and Vasanthakumar *et al.* (2001) in rabbits. However, Christopher *et al.* (1976) have reported intensive fatty changes in liver and kidneys at 30% NSC level. Tissue specific inhibition of detoxifying enzymes in liver, lungs and kidneys has been reported in rats following administration of Azadirachtin (Mehboob *et al.* 1995). Therefore it appears that degenerative changes in liver and kidneys have been due to failure to detoxify the toxic principles of NSC.

Proventriculus showed denuded mucosa with diffuse leucocytic infiltration (Fig. 3) which extended up to glandular acini in a few experimental chicks. There was sero-fibrinous exudate in submucosa along with cellular debris in glandular acini. The lesions appeared almost similar in all the groups and continued till the end of the experiment. However, Christopher *et al.* (1976) reported catarrhal proventriculitis at 30% NSC level in cockerels.

Microscopically, lungs did not show prominent lesions except for congestion and haemorrhages. In a few chicks, there was increased number of goblet cells in the bronchiolar mucosa which was thrown into folds and projected into the lumen due to underlying lymphoid aggregates. In pancreas, mild congestion and oedema in the pancreatic lobes was evident in groups 3 and 4 chicks during early stages, whereas in later stages, degenerative changes in acinar cells and islets of Langerhans became appreciable (Fig. 4). On 35 DPT, foci of lymphocytic infiltration were observed in interlobular tissue and in few cases, excessive fibrous tissue was evident extending into interlobular tissue. Few necrotic foci with lymphocytic infiltration became evident between glandular acini at 42 DPT and onwards. There appears to be no published report regarding microscopic lesions is the lungs and pancreas following NSC feeding.

However, there were no appreciable microscopic changes in gizzard, oesophagus, spleen, thymus and trachea in any of the group at any stage of experiment.

The results of the present study are indicative of mild degenerative changes in different visceral organs. The histopathological changes in brain, bursa of Fabricius, heart, proventriculus, lungs and pancreas appear to be the first report in broiler chickens following NSC feeding. The effects appear to be due to some toxic ingredient present in the raw *neem* seed-cake. This toxic ingredient needs to be identified and isolated from *neem* seed-cake before mixing it in the poultry ration.

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Toxicity studies on *Prosopis juliflora* in goats—Haematobiochemical and pathological profile

JYOTI MISRI[1], V S VIHAN[2] and ASHOK KUMAR[3]

Central Institute for Research on Goats, Makhdoom, Uttar Pradesh 248 122 India

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ABSTRACT

Prosopis juliflora mature pods were experimentally fed to 18 goats, which were allocated to 3 groups of 6 animals each. Animals in group A were kept as untreated controls. Animals in group B were fed *P. juliflora* pods @ 400 g/animal/day and group C were given exclusively *P.juliflora* mature pods for 4 days. On an average the animals ate 508 g dry pods of *P.juliflora* in group C. Hybrid Napier grass was fed as basal feed. Partial anorexia, depression, salivation, twitching, dehydration and bloody diarrhoea were observed in affected goats. In *P.juliflora* – treated goats, there was a significant ($P<0.01$) decrease in haemoglobin (8.85±0.22 g/dl in treated group C and 10.53±0.09 g/dl in control group A, ruminal pH 5.23±0.015 in treated group C and 7.09±0.01 in control group A, ruminal bacterial count $13.80\times10^9\pm0.55$/ml in treated group C and $20.80\times10^9\pm0.02$ in control group A and total disappearance of the ruminal protozoa to $1.360\times10^5\pm0.27$.) There was a significant decline in blood pH, increase in blood urea nitrogen (24.79±0.79 mg/dl in group C and 14.84±0.04mg/dl in untreated control group) with increased serum amylase and aspartate –amino transferase activity. Histopathological studies revealed necrotic lesions in the liver with bile duct hyperplasia. Degenerative changes were observed in renal tubules and lymphnodes revealed rarefaction of lymphoid tissue.

Key words: Goats, Microbial profile, Pathology, Rumen

Prosopis juliflora locally known as *vilayati babool* is a small tree belonging to leguminosae family. It is largely available in northern western (hot dry) region of the country, where it is usually grown for afforestation of dry areas. Due to scarcity of green fodder in lean periods, goat being a natural browser eat these plants along with pods, as a source of nutrition. The mature dry pods of *P.juliflora* especially in the month of May-June, when eaten by goats lead to apparent impairment in digestion, nervous manifestations, deleterious effects on body organs and at times death (Clarke *et al.* 1981). It also at times leads to cyanide poisoning in cattle when mixed with sugarcane, as feed. Due to paucity of information on the effect of feeding of *P.juliflora* pods to goats, the present study was undertaken to assess its effect on the microbial profile of rumen, haemato-biochemical, gross and histopathological changes.

MATERIALS AND METHODS

Non-descript adult goats (18) were taken and 3 groups were formed. Group A (normal ration) served as untreated control. Animals of group B were fed mature dry pods @400g/day/animal and hybrid Napier grass, group C were only fed pods *ad lib*. The feeding was done for 4 days. Experimental animals were kept on fast for 24hr prior to feeding of *P.juliflora* with free access to water. Before the start of feeding experiment, blood profile of rumen liquor were recorded as 0 day value, thereafter similar observations were made on days 1, 2, 3, 5 and 7 post feeding.

The pH of the rumen liquor was recorded with digital pH meter. The rumen liquor was collected by stomach tube method and strained rumen liquor was used for various biochemical and microbial indices, viz. total and differential count of rumen protozoa (Naga and Shazly 1969) and total bacterial count (Cruikshank 1965). Blood samples were analyzed for haemoglobin (Jain 1986), biochemical constituents viz. blood urea nitrogen (Ormsby 1942), serum amylase (Street and Close 1956), serum aspartate aminotransferase (Reitman and Frankel 1957) and blood serum glucose by 'O' toluidene method (Hultman 1954).

Detailed systematic necropsy of each animal was done. All the organs of 3 goats sacrificed by GPT (goat products technology lab.) /died goats were first examined *in situ* and visible patho-anatomical lesions were noted. Small pieces of tissues about (10 mm × 5mm) from various organs were collected in 10% formal saline for detailed histopathological examination. The data were analysed statistically using

Present address: [1]Scientist (Senior Scale), Policy and Prospective Planning, ICAR, Krishi Bhawan, New Delhi 110 001.

[2]Head, [3]Senior Scientist, Division of Goat Health.

complete randomized block design (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Affected animals showed emaciation, ruminal acidosis, with distended abdomen, dilated pupils, anxious look and recumbency. Skin coat was rough and dry with loss of elasticity. Mucous membranes were pale yellow. Liver was moderately enlarged with rounded edges. Kidneys were pale, enlarged, edematous and congested. Lungs were pale, emphysematous and in some cases consolidation of right apical and diaphragmatic lobes were seen with pleural adhesions. Thyroid, pancreas and lymphnodes were congested and revealed degenerative changes. Histopathologically liver revealed necrotic changes and coagulative necrosis with cellular infiltration. Bile ducts were hyperplastic and rumen showed golden yellow inspissated bile pigment. Kidneys and lymph nodes revealed early degenerative changes with areas of haemorrhages in the tubules. Similar hepatic necrosis and degenerative changes have been reported by Jensen (1954). However, the degenerative changes and depletion of lymphoid tissue as seen in our study suggest the toxic effects of *P.juliflora* feeding.

Tabosa *et al.* (2000) found neuronal vacuolation of the trigeminal nuclei in goats caused by ingestion of *P. juliflora* pods (mesquite beans).Histologic lesions were characterized by fine vacuolation of the pericaryon of neurons from the trigeminal nuclei. Denervation atrophy of the masseter, temporal, hypoglossus, genioglossus, styloglossus, medial pterygoid and lateral pterigyoid muscles were seen resembling to (Taboosa *et al.* 2000). They opined that the feeding of *P.juliflora* pods caused selective toxicity to neurons of some cranial nerve nuclei.

The results of the haematological observations and microbial profile studies of the rumen are summarized in Table 1 and serum biochemical changes in Table 2. The ruminal pH dropped significantly (P<0.01) on day 1 with a further drop up to day 3. From day 4 onwards the pH began to increase slowly but it was still lower than the normal values. The colour of the rumen liquor changed from greenish brown to light grey, probably due to increased acid content as reported earlier by Sen *et al.* (1982). Rumen pH decrease may be due to faster fermentation by amylolytic bacteria leading to lactic acid production. Total and differential counts of ruminal protozoa were within normal range at zero day of observation. Complete disappearance of ruminal protozoa on day 1 of experiment was noticed in some animals and lower values were maintained till day 3. The protozoa were predominantly *Entodinium* spp. (80%) which appeared in smaller number on day 7. There was also significant decrease (P<0.01) in rumen bacterial population on day 1 which started improving from day 3 when it was $14.86 \times 10^9 \pm 0.09$/ml. High concentration of lactic acid and low ruminal pH could lead to disappearance of rumen protozoa. The reappearance of ruminal protozoa from day 3 onwards could be synchronized with the improvement in ruminal pH. The reduction in total bacterial count may be due to destruction of cellulolytic bacteria at low pH in ruminants (Dunlop 1972).

A gradual but significant decrease in blood pH was observed from day 1 to 3. However, from day 4 onwards, it showed a rising trend, which was nearly normal on day 7 post-feeding of *P.juliflora.* Significant increase in blood urea nitrogen (P<0.01) up to day 3 was observed and thereafter it remained almost constant. Increased concentration of blood urea nitrogen may be due to reduced renal blood flow and fall in arterial blood pressure, which in turn could lead to abnormal renal function. This could also be correlated to the evidence of degenerative and necrotic changes in kidney tubules observed histopathologically. There was significant increase in amylase activity on day 1 compared to normal values on zero day. The decline was, however, observed after day 3, again elevated amino aspartate transferase was recorded on day 1 till day 3 in comparison to normal untreated group

Table 1. Haematological and ruminal microbial status in experimental *P.juliflora* feeding in goats (mean±SE)

Entities treatment	Hb g/dl	Rumen pH	Total bacterial count/ ml	Total protozoal count/g of ruminal ingesta
A	10.53^{a}±0.09	7.08^{a}±0.01	$20.77^{a}10^{9*}$0.01	$4.21^{a} \times 10^{9}$±0.01
B	9.18^{b*}0.20	5.31^{b*}0.14	$14.15^{b} \times 10^{9*}$0.52	$1.40^{b} \times 10^{9}$±0.26
C	8.85^{c*}0.22	5.23^{c}±0.15	$13.79^{c} \times 10^{9}$±0.54	$1.35^{c} \times 10^{9}$±0.26
Days				
0	10.96±0.10	7.07±0.01	$20.68 \times 10^{9*}$0.16	$4.19 \times 10^{5*}$0.01
1	10.32±0.09	5.47±0.28	$15.88 \times 10^{9*}$0.84	$1.39 \times 10^{5*}$0.48
2	9.35±0.20	5.38±0.28	15.31×10^{9}±1.02	1.39×10^{5}±0.48
3	8.79±0.35	5.57±0.27	14.86×10^{9}±0.97	1.42×10^{5}±0.48
5	8.63±0.30	5.80±0.23	15.18×10^{9}±0.96	2.61×10^{5}±0.28
7	9.04±0.20	5.96±0.20	15.50×10^{9}±0.97	2.92×10^{5}±0.23

Means with different superscripts vary significantly (P< 0.01) ; between day the difference is significant with each other (P<0.01); (0,1.2,3,5,7 days) denotes the values of all the animals on that day.

Table 2. Biochemical profile in blood in experimental *P.juliflora* feeding in goats (mean±SE)

Entities treatment	Blood pH	Blood sugar	Serum amylase (u/litre)	Aspartate amino transferase (Rfu/dl)	Blood urea nitrogen (mg/dl)
A	7.40[a]±0.00	59.720[a]±0.20	523.05[a]±0.74	62.59[a]±0.16	14.84[a]±0.04
B	7.24[b]±0.02	77.08[b]±0.20	566.57[b]±9.74	88.85[b]±2.11	24.00[b]±0.77
C	7.31[c]±0.01	79.83[c]±2.09	567.89[c]±10.25	90.26[b]±2.42	24.78[c]±0.79
Days					
0	7.41±0.00	59.45±0.31	524.22±1.43	63.06±0.16	14.69±0.09
1	7.33±0.01	82.72±3.95	532.89±1.92	100.11±4.87	20.25±0.93
2	7.30±0.02	79.21±3.21	639.25±19.83	88.34±2.80	22.69±1.34
3	7.23±0.03	76.40±2.89	545.12±4.23	84.57±1.99	23.18±1.41
5	7.30±0.02	70.06±1.83	538.55±3.02	79.23±1.27	23.35±1.45
7	7.31±0.02	65.42±0.99	534.98±2.74	78.09±1.07	23.07±1.41

Means with different superscripts vary significantly ($P< 0.01$); between day the difference is significant with each other ($P<0.01$) ; (0,1,2,3,5,7 days) denotes the values of all the animals on that day. ABC in both the tables designate the three different groups with 3 treatments.

values. Serum amylase activity increased markedly on day I and remained higher at various intervals. The significant increase in amino-aspartate transferase activity may be due to hepatocellular damage following acidosis as evinced histopathologically. Increased blood glucose and serum amylase in the present study could be attributed to degenerative changes observed in pancreas.

Thakur (1991) isolated 2 antigenic glycoproteins from the pollen of *P. juliflora* using DEAE-cellulose ion exchange chromatography. The molecular weight of these 2 glycoproteins were 20 000 and 10 000 by gel filtration on sephadex G-75.In ELISA the polyclonal antisera raised in rabbit showed strong binding affinity with glycoprotein of molecular weight 20 000. The results indicated that the 2 glycoprotein fractions are not antigenically identical. There are reports of isolation of julifloricine an alkaloid by Aqeel *et al.* (1989) from *P. juliflora.* This alkaloid was found inactive against fungi (up to dose of 10 micro g/ml) and viruses.

The study has shown that *P.juliflora* pods when taken in unlimited quantities are toxic and produce acidosis leading to impairment in body tissues and organs. Prolonged and continuous feeding can be fatal and could lead to sudden deaths also. The observations further showed that immune status of the animals is affected as evident histopathologically with the diminished lymphoidal tissue.

The toxic effects of *P.juliflora* probably appears to be similar to cyanide poisoning as reported by Clark (1981) in cattle when *P.juliflora* pods were fed to cattle along with sugarcane. The toxins could not be identified. There are earlier reports of some allergens found in the *P.juliflora* pods which probably cause hypersensitivity reaction and add to the cumulative toxic effects exerted by its consumption.

There is thus further need to study this area in depth and bring to the forefront the negative side of the unconventional /accidental feeding of *P.juliflora* pods, which has a scope of being utilized as an alternate unconventional feed because they are cheap and easily available in extreme summer especially in dry zones.

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Serum progesterone and estradiol 17ß concentration in relation to superovulatory response in embryo donor (Sahiwal) and conception rate in embryo recipient (crossbred) cattle

SHIV PRASAD[1], S N MAURYA[2] and V P VARSHNEY[3]

Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttaranchal 263 145 India

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ABSTRACT

Serum progesterone (P_4) and estradiol 17ß levels were measured in embryo donor (Sahiwal) and recipient (crossbred) cows in relation to superovulatory response and conception rate respectively. Sahiwal cattle (19) were superovulated with equine and porcine follicle stimulating hormone and they were divided into 3 groups on the basis of super ovulatory response (good, fair and non responders). The superovulatory response was better in the animals having lower levels of progesterone on the day of initiation of superovulatory treatment compared to those having higher levels of progesterone on the day of initiation of gonadotrophin treatment. The levels were 2.27±0.17, 2.51±0.15 and 3.08±0.22 ng/ml in good, fair and non-responder cattle respectively. The level of estradiol 17ß started increasing gradually from the day of initiation of gonadotrophin treatment and reached peak level on the day of superovulatory estrus. The superovulatory response was better in the animals having higher levels of estrogenic peak on the day of super heat compared to other groups having comparatively lower levels of estrogenic phase on super heat. The levels of estradiol 17ß in good, fair and non-responders on the day of super heat were 28.81±5.12, 14.80±1.16 and 10.08±1.23 pg/ml respectively. The level of P_4 was similar to day 7 post E T in embryo recipient animals but it differed significantly ($P<0.05$) after day 7 post ET In pregnant animals it continued to rise while in non-pregnant it came down on day 21 post E T. Estradiol 17ß in pregnant cattle came down to 4.76±0.40 pg/ml from 5.08±0.40 on day 7 post E T while it started rising from day 7 post E T gradually in non-pregnant cattle from 5.78±0.32 pg/ml to 6.08±0.47 pg/ml on day 14 post E T

Key words: Progesterone, Estradiol 17ß, Superovulatory heat, Pregnancy

Progesterone concentration directly reflects the function of corpus lutuem, and is a precise indicator of ovarian function. Plasma progesterone concentration in cattle varies from 0.1 to 0.8 ng/ml at the time of estrus (Diaz *et al.* 1986, Saxena and Gupta 1992). Subsequently, it increases to a mean value of 4.5 ng/ml around days 9 to 16 followed by a decline to its basal level (0.4±0.1 ng/ml) by day 18-21 of estrous cycle. Significant relationship was reported between progesterone profile at the time of gonadotrophin treatment initiation to ovarian response, total and transferable embryos (Yadav *et al.* 1986, and Goto *et al.* 1988).

The concentration of estradiol 17ß increases 4 to 5 days before estrus and touches the peak level at estrus (Dobson and Dean 1974). The increase in estradiol - 17ß at estrus was 2-6 fold above mid-luteal phase value (Henricks *et al.* 1971, Kotwica and Williams 1982). The blood levels of estradiol 17ß concentration was 3-4 times higher at estrus in super ovulated than that in non-superovulated animals (Booth *et al.* 1975). In the present study the progesterone and estradiol 17ß were evaluated in relation to superovulation in Sahiwal cattle and conception following embryo transfer in crossbred cattle.

Present address: [1]Associate Profesor, [2]Professor and Head, Department of Animal Reproduction, Gynaecology and Obstetrics; [3]Senior Scientist and I/C NRL, IVRI, Izatnagar 243 122.

MATERIALS AND METHODS

The embryo donor (Sahiwal) and recipient (crossbred) cows were selected from the livestock centre of G.B. Pant University of Agriculture and Technology, Pantnagar. Animals were selected on the basis of attributes of reproductive records maintained at the LRC and twice pre-rectal examination of the animal at the time of estrus.

Embryo donor animals were subjected to superovulatory treatment by using FSH-P and FSH-E and subsequently divided into 3 groups i.e. good responders, fair responders and non-responders. On the basis of superovulatory response, dose and regimen used was as per following schedule. The

blood samples were collected from embryo donor and recipient animals as per following schedules.

Superovulatory treatment with FSH-P

Day of treatment	Donors	Recipients
-03	Lutalyse, 25mg, I/M	Lutalyse, 25 mg, I/M
0	Estrus	Estrus
10	Folltropin, 65mg, I/M (M)	–
	Folltropin, 65mg, I/M (E)	
11	Folltropin, 55mg, I/M (M)	Lutalyse, 25 mg I/M (E)
	Folltropin, 55mg, I/M (E)	
	Folltropin, 45mg, I/M (E)	
12	Folltropin, 45mg, I/M (M)	–
	Lutalyse, 25mg, I/M (M)	
13	Folltropin, 35mg, I/M (M)	–
	Folltropin, 35mg, I/M (E)	
14	Estrus and AI	Estrus, no breeding

Superovulatory treatment with FSH-E

Day of treatment	Donors	Recipients
-03	Lutalyse, 25mg, I/M	Lutalyse, 25 mg, I/M
0	Estrus	Estrus
10	FSH, 7.5mg, I/M (M)	
	FSH, 7.5mg, I/M (E)	–
11	FSH, 6.5 mg, I/M (M)	
	FSH, 6.5 mg, I/M (E)	Lutalyse, 25 mg I/M (E)
12	FSH, 6.0 mg, I/M (M)	
	Lutalyse, 25 mg, I/M (M)	
	FSH, 6.0 mg, I/M (E)	–
13	FSH, 5.0 mg, I/M (M)	
	FSH, 5.0 mg, I/M (E)	–
14	Estrus and AI	Estrus, no breeding

Blood (15) was collected from jugular vein and serum was separated. The serum was stored at -20°C till analysis. Serum samples were analyzed for progesterone (P_4) and estradiol 17ß by radioimmunoassay (RIA) using RIA kits. The values were calculated from the standard curve. The data generated

Sampling schedule of embryo donor and recipient animals

Samples	Day of collection
Embryo donor	
I	-3 day (day of first PG treatment)
II	0 day (day of estrus)
III	+ 2 day post estrus
IV	+ 5 day post estrus
V	+10 day (day of initiation of FSH treatment)
VI	+12 day (day of second PG treatment)
VII	+13 day (Last day of FSH treatment)
VIII	S_0 (day of superovulation)
IX	S_3 (third day of superovulation)
X	S_7 (seventh day of superovulation)
Embryo recipient	
I	Day of ET
II	Day 4 post E.T.
III	Day 7 post ET
IV	Day 14 post ET
V	Day 21 post ET
VI	Day 28 post ET

during the study was analyzed by two factors CRD (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The level of progesterone prior to prostaglandin injection was 1.58±0.12ng/ml in good responder animals (> 6CL), whereas, 2.42±0.45 and 2.34±0.39 ng/ml in fair and non-responder animals. It dropped around 1ng/ml on the day of estrus in all the groups, which is quite obvious that prostaglandin causes lysis of corpus luteum and subsequent drop in progesterone concentration in the blood serum. Rajamahendran *et al.* (1976) have also reported progesterone level less than 1 ng/ml during first 2 days of the cycle, it increased rapidly over 4th to 12th day and reached a peak level of 5.2±1.1ng/ml on day 14 of the cycle. Thereafter, the level declined to 2.6±0.6ng/ml on day 16 and to 0.40±0.1 ng/ml on day 21 of the cycle. In this study the level rose up to

Table 1. Serum progesterone levels (ng/ml; mean ± SE) in embryo donor cows

Donor response			Days from estrus									
	CL	AF	-3	0	2	5	10	12	13	S_0	S_3	S_7
Good	11.66	4.00	1.58^{Aacd}	0.75^{Aa}	1.39^{Aacd}	2.12^{Abcd}	2.27^{Abc}	2.06^{Abcd}	1.64^{Bacd}	0.87^{Aad}	2.31^{Abc}	4.61^{Ac}
	±1.91	±1.53	±0.12	±0.14	±0.18	±0.13	±0.17	±0.42	±0.12	±0.12	±0.20	±0.14
Fair	3.6	1.8	2.42^{Ab}	0.98^{Aa}	1.48^{Aab}	1.81^{Aab}	2.51^{Ab}	3.23^{Ac}	3.11^{Ac}	0.84^{Aa}	2.29^{Ab}	3.17^{Ac}
	±0.22	±0.14	±0.45	±0.08	±0.08	±0.22	±0.15	±0.17	±0.30	±0.04	±0.22	±0.37
Non-responders	1.5	1.00	2.34^{Abcd}	0.98^{Aa}	1.27^{Aab}	2.60^{Acde}	3.08^{Acde}	3.33^{Ade}	3.49^{Ade}	1.18^{Aab}	2.05^{Aabc}	4.12^{Af}
	±0.08	±0.08	±0.39	±0.10	±0.12	±0.23	±0.22	±0.54	±0.58	±0.14	±0.20	±1.39

Cd_3 at 5% = 1.253; F value: a = 2.851; b = 13.282**; a × b = 1.135 ns. Means within a row with different small superscript are significantly different (P<0.05); means within a column with different capital superscript are significantly different (P<0.05).

Table 2. Serum progesterone levels (ng/ml; mean ± SE) in embryo recipient cows

Recipients	Day of ET	Day 4 post ET	Day 7 post ET	Day 14 post ET	Day 21 post ET	Day 28 post ET
Pregnant	5.19^{Aa}	5.46^{Aa}	6.13^{Aa}	7.34^{Aa}	7.75^{Aa}	8.06^{Aa}
	±0.41	±0.38	±0.41	±0.73	±0.67	±0.75
Non-pregnant	5.40^{Aa}	5.57^{Aa}	6.67^{Aa}	5.16^{aB}	4.72^{aB}	2.76^{aB}
	±0.53	±0.46	±0.20	±0.90	±0.89	±0.70

Cd_3 at 5% = 1.773; F value: a = 19.77**; b = 1.24ns; a × b = 6.83**.Means within a row with different small superscript are significantly different (P<0.05); means within a column with different capital superscript are significantly different (P<0.05).

day of initiation of gonadotrophin treatment (D-10) in good responder animals (Table 1) and it remained high up to day of prostaglandin treatment (D-12). Progesterone concentration dropped below 1 ng/ml on the day of superovulatory estrus in 2 groups but did not come down sharply in third group, which remained at 1.18±0.14 ng/ml (Table 1). This type of relationship in the level of progesterone was also reported by Lindsell *et al.* (1986) and Kweon *et al.* (1987). Saumande (1980), Jensen *et al.* (1982) and Greve *et al.* (1983). They have also reported the level of P_4 more than 1 ng/ml in some animals. Ullah *et al.* (1992) and Tamboura *et al.* (1985) have reported, respectively, in cows and buffaloes that there is no correlation between the higher level in progesterone on the day of initiation of FSH treatment and embryo recovery or quality. In this study also it was found that in fair and non-responder animals the higher levels of progesterone on the day of initiation of FSH treatment was not correlated with recovery and quality of embryo. These findings fitted well with the above authors. Gradella *et al.* (1994) reported that plasma progesterone concentration on the day of embryo collection was positively correlated with number of corpora lutea palpated (0.80 and 0.67) respectively. In our study the corpus luteum were more in the first group which corresponds well with the levels of progesterone. The similar pattern was seen in fair responder group but not in non-responder group. The level of progesterone was significantly different after day 14 post ET (Table 2). Rao *et al.* (1997) also reported similar findings of plasma progesterone concentration in 47 *Bos indicus* × *Bos taurus* embryo recipient cattle and they observed that the level of progesterone did not vary. On day of estrus, day 7 of the estrous cycle in pregnant and non-pregnant cattle, whereas, the blood progesterone concentration on day 21 was 12.2 ng/ml in pregnant females vs 0.82 ng/ml for non-pregnant females.

In this study, the levels of estradiol 17ß on the day of estrus and on the 5th, 10th and 13th of the estrous cycle were 6.94±0.78, 4.89±0.54, 5.15±0.63 and 9.24±1.41 pg/ml, respectively, in good responder animals. Present findings are

Table 3. Serum estradiol 17ß levels (pg/ml; mean ± SE) in embryo donor cows

Donor response	CL	AF	Days from estrus									
			-3	0	2	5	10	12	13	S_0	S_5	S
Good	11.66	4.00	5.61^{Aa}	6.94^{Aab}	5.86^{Aab}	4.89^{Aa}	5.15^{Aa}	6.80^{Aab}	9.24^{Abc}	28.81^{cC}	16.34^{dB}	12.37^{cB}
	±1.91	±1.53	±0.43	±0.78	±0.56	±0.63	± 0.63	±0.23	±1.41	±5.12	±2.47	±1.09
Fair	3.6	1.8	4.61^{Aa}	6.29^{Aab}	5.34^{Aa}	6.31^{Aab}	6.14^{Aab}	7.35^{Aabc}	8.80^{Abc}	14.80^{dB}	10.41^{cA}	8.03^{abcA}
	±0.22	±0.14	± 0.61	±0.54	±0.70	±0.34	±0.06	±0.33	±0.24	±1.16	±0.79	±0.59
Non-responders	1.5	1.00	4.19^{Aa}	5.56^{Aab}	4.99^{Aab}	4.91^{Aab}	5.38^{Aab}	5.25^{Aab}	6.22^{Aabc}	10.08^{dA}	7.78^{bcA}	5.56^{abA}
	±0.08	±0.08	±0'.41	±0.47	±0.55	± 0.47	±0.57	±0.58	±0.38	±1.23	±0.78	±0.37

Cd_3 at 5% = 3.438; F value: a = 29.192**; b = 32.192**; a × b = 6.454**; means within a row with different small superscript are significantly different (P<0.05); means within a column with different capital superscript are significantly different (P<0.05).

Table 4. Serum estradiol 17ß levels (pg/ml; mean ± SE) in embryo recipient cows

Recipients	Day of ET	Day 4 post ET	Day 7 post ET	Day 14 post ET	Day 21 post ET	Day 28 post ET
Pregnant	5.64^{Aa}	5.08^{Aa}	4.76^{Aa}	4.65^{Aa}	4.48^{Aa}	4.16^{Aa}
	±0.37	±0.40	±0.40	±0.40	±0.35	±0.31
Non-pregnant	5.89^{Aa}	5.639^{Aa}	5.78^{Aa}	6.08^{aB}	5.94^{aB}	6.82^{aB}
	±0.27	±0.25	±0.32	±0.47	±0.49	±0.70

Cd_3 at 5% = 1.169; F value: a = 26.99**; b = 0.450ns; a × b = 2.05ns. Means within a row with different small superscript are significantly different (P<0.05); means within a column with different capital superscript are significantly different (P<0.05).

similar to the Purohit *et al.* (2000). The level of estradiol on the day of superovulatory estrus was twice to the level of estradiol on the day of natural estrus. Henricks *et al.* (1971) and Booth *et al.* (1975) studied estradiol 17ß concentrations in superovulated cows and they found that it increased 3-4 times higher at estrus in super ovulated than in non-super ovulated animals. Estradiol 17ß concentration during super ovulatory treatment had relation with the number of follicles ovulated (Table 3). Similar relationship was also recorded by Saumande (1978) and Lindsell *et al.* (1986). There was no significant variation in the level of estradiol 17ß in pregnant and non-pregnant animals up to day 7 post ET (Table 4). The level changes beyond day 14 post ET and estradiol goes down on day 14 post ET in pregnant animal, and it has risen in non-pregnant. In the present study the level of estradiol 17ß has not fallen sharply on day 14 post ET which could be due to incomplete regression of corpus luteum (Table 4). Thus, significantly ($P<0.05$) higher level of estradiol 17ß on super ovulatory estrus and lower level of progesterone on the same day in good responder animals resulted in better super ovulatory response compared to other groups.

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Age correlated histoenzymic distribution of acetyl cholinesterase and non-specific esterase in the ovary of Indian buffalo (*Bubalus bubalis*)*

R L BHARDWAJ[1] and K S ROY[2]

Punjab Agricultural University, Ludhiana, Punjab 141 004 India

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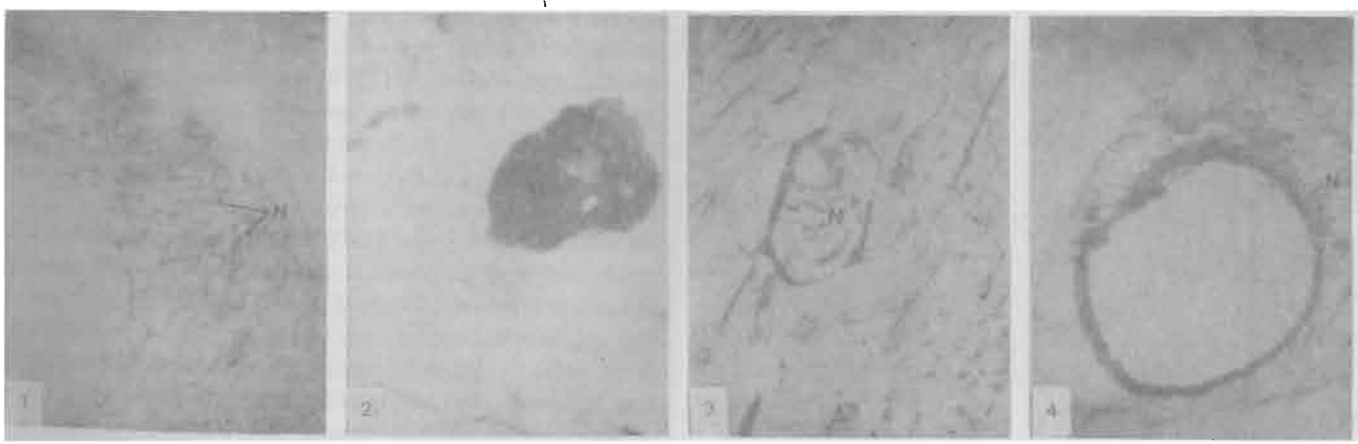
ABSTRACT

The study was conducted on the ovaries of 46 (13 neonatal, 3 prepubertal, 22 cyclic, and 8 pregnant) healthy Murrah female buffaloes. The fresh unfixed ovarian tissues were collected in the liquid nitrogen and cryostal sections of 10 µm thickness were obtained and incubated for acetylcholinesterase (AChEase) and non-specific esterases (NSE). AChEase positive nerve fibres and its terminal and ganglion were seen in the buffalo ovary. Fine nerve terminal seen reaching up to basement membranc of ovarian follicles. Strong NSE activity was seen in the ovarian follicles, luteal cells, nerve fibres and interstitial glands.

Key words: AChEase, Age, Buffalo. NSE, Ovary

The buffalo is known for the poor reproductive performance such as silent heat, low conception rate and long calving interval, thus causing heavy economical losses to the farmers. Buffalo ovary is characterised by low number of primordial follicle reserve and high incidence of follicular atresia to the extent of approximate 65% (Guraya 1979, Danell 1987, Deloos *et al.* 1991, Bhardwaj and Roy 1999). However, detail of age related histoenzymological studies on the ovarian follicles during postnatal development, estrus cycle and pregnancy are scanty and no systematic studies have been reported on buffalo ovary. The present study is aimed to elucidate the histoenzymic distribution of AChE ase and NSE in the ovary of buffaloes of various age groups.



Figs 1-4. Photomicrograph of ovary from neonatal buffalo calf showing: **1**. Acetylcholinesterase activity in nerve fibres (N) of cortex and in the wall of graafian follicle. Thiocholine method × 18; **2.** Ganglionic cells (G) with AChEase activity. Thiocholine method × 70; **3.** AChEase activity in nerve fibres (N) in perivascular area. Thiocholine method × 70; **4.** Photomicrograph of ovary from cyclic buffalo showing activity of AChEase in the nerve fibres (N) around large graafian follicle as well as in the stromal tissue. Thiocholine method × 70.

MATERIALS AND METHODS

The study was conducted on the ovaries of 46 buffaloes (13 neonatal, 3 prepubertal, 22 cyclic and 8 pregnant). The fresh unfixed ovarian tissues were collected in liquid nitrogen

*Part of Ph.D. Thesis

Present address: [1]Associate Professor, HPKVV, Palampur 176 062.

[2]Professor and Head, Department of Anatomy and Histology.

Table 1. Age related histoenzymic distribution in the ovary of buffalo

Enzyme/ ovarian components	Secondary							Graafian tertiary				Atretic									
	Suface epith-elium	Tunica albug-ina	Cortical stroma	Pnmordial and primary	Theca	Membrane granulosa	Zona pellu-cida	Oocyte	Theca	Membrane granulosa	Zona pellu-cida	Oocyte	Theca	Membrane granulosa	Zona pellu-cida	Oocyte	Interstitial gland cells	Medullary strotma	Blood vessels	Neres ganglion	Rete ovarii	Hilar glands	Corpus luteum
AChEase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+++	0	0	0
NSE	0	0	0/	+	0	+	0	0	+	+	0	0					+++	0/+	+	+++	++	0	+
	/	/	+	+		+											+						+
	+	+		+		+																	+
AChEase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				++	0	0	0
NSE	0	0	0/	+	+	+	0	0	+	+	0	0	+	+	0	0	+++	0/+	+	+++	++	0	+
	/	/	+	+	+	+			/	/			/	/			+		+	+			+
	+	+		+	+	+			+	+			+	+									+
AChEase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0
NSE	+	+	0/	+	0	+	0	0	0	+	0	0	0	+	0	0	+	0/+	+	++	+	0	0
			+		/				+				/										
				+				+															
AChEase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0
NSE	+		+	+	+	+	0	+	+	+	0	+	+	+	0	+	+++	+	+	+++	‡	0	0
																				/++	+		
																				++			

0= Negative; O/+= negligible, += moderate; +++= strong; +++= intense.

for histoenzymic demonstration of AChEase and NSE. The tissues were put to cryostat microtome to obtain sections of 10 µm thickness on clean glass slides and were incubated for demonstration of AChEase (El-Badawie and Schenk 1967) and NSE (Barka and Anderson 1963).

RESULTS AND DISCUSSION

There was strong to intense AChEase activity in the neuronal elements of the buffalo ovary (Table 1). Strongly positive nerve fibres were more in the perivascular connective tissue (Fig:3). Fine nerve terminals were seen innervating the follicular wall (Fig.1) reaching almost to the membrana granulosa cells (Figs 1,4). At location ganglionic cells were also strongly positive for AChEase (Fig. 2) Roy and Saigal (1985) also demonstrated strong activity in the neuronal elements of the sheep ovary. The AChEase plays an important role in the transmission of nerve impulses. Burden and Lawrence (1978) described in detail the AChEase activity in the ovary, and suggested that catecholamines stimulate the progesterone production by corpora lutea. The presence of AChEase in lutein cells of cyclic and pregnant buffalo may presumably help in steroidogenesis through the hydrolysis of acetylthiocholine resulting in the formation of acetic acid to be used subsequently for the progesterone synthesis.

The non-specific esterase activity in the surface epithelium, tunica albuginea and ovarian cortex was negligible to weak in the ovaries of buffalo in all age groups (Table 1). Theca cells of the various types of follicles showed varied amount of NSE activity. It was moderate and strong in growing follicles in neonatal and cyclic animals respectively. The atretic follicles showed decreasing trend of activity in the theca cells in all the animals except pregnant ones. Bhattacharya and Saigal (1990) reported moderate to strong activity in the theca cells of goat ovarian follicles.

The NSE activity in the membrane granulosa was weak to moderate in neonatal and weak in prepubertal and moderate to strong in the cyclic and weak to strong in pregnant ones. Bhattacharya and Saigal (1990) in goat reported moderate, strong and weak activities in secondary, tertiary and atretic follicles respectively. The NSE activity in the zona pellucida and oocyte could not be observed in the ovarian follicles of prepubertal, cyclic and pregnant animals but weak activity was observed in the oocyte of the neonatal group.

Interstitial gland cells showed intense activity of NSE in pregnant and cyclic groups, whereas, it was weak in the prepubertal and strong in neonatal ones. Bhattacharya and Saigal (1990) also reported moderate to strong activity in the interstitial gland cells of the ovary of goat. It is believed that NSE participate in the lipid metabolism and leads to steroidogenesis. Blood vessels showed weak to moderate activity of NSE while nerves showed moderate to intense activity. The rete ovarii showed moderate activity in prepubertal and adult, while it was strong in neonatal animals.

Intense activity of NSE was observed in the luteal cells in buffalo ovary as reported earlier in sheep (Bhattacharya and Saigal 1990). The enzyme plays its role in lipid metabolism (Talanti 1958). It breaks the lipid membrane secretory granules and activates the secretion (Leonieni and Rechard 1972).

It is concluded from above study that AChEase and NSE may be participating in production of steroid hormones from buffalo ovary.

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Age correlated histoenzymic distribution of reduced nicotinamide adenine dinucleotide diaphorase and reduced nicotinamide adenine dinucleotide phosphate diaphorase in the ovary of Indian buffalo (*Bubalus bubalis*)*

R L BHARDWAJ[1] and K S ROY[2]

Punjab Agricultural University, Ludhiana, Punjab 141 004 India

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ABSTRACT

The study was conducted on the ovaries of 46 (neonatal 13, prepubertal 3, cyclic 22, and pregnant 8) normal healthy Murrah buffaloes. The fresh ovarian tissues were collected in the liquid nitrogen for the histoenzymic demonstration of enzymes. The unfixed frozen tissues were put to cryostat microtome at -20°C to obtain sections at 10μm on clean glass slides and incubated for reduced nicotinamide adenine dinucleotide diaphorase (NADH- diaphorase) and reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH- diaphorase) activity. The enzymes showed varied activity of NADH and NADPH-diaphorase in the different components of the buffalo ovaries and in the graafian follicles.

Key words: Buffalo, Diaphorase, Ovary

The buffalo is known for the poor reproductive performance such as silent heat, low conception rate and long calving interval thus causing heavy economical losses to the farmers. Low number of primordial follicle reserve characterizes buffalo ovary and high incidence of follicular atresia to the extent of approximate 65% (Guraya 1979, Danell 1987, Deloos *et al.* 1991, Driancourt *et al.* 1991, Bhardwaj and Roy 1999). However, detail of age related histoenzymological studies on the ovarian follicles during postnatal development, estrus cycle and pregnancy are scanty and no systematic studies have been reported on buffalo ovary. Hence to understand the follicular development and atresia during postnatal development, estrus cycle and pregnancy is prerequisite for the improvement of buffalo production and reproduction. The present study is aimed to elucidate the histoenzymic distribution of NADH diaphorase and NADPH-diaphorase in the ovary of various age groups.

MATERIALS AND METHODS

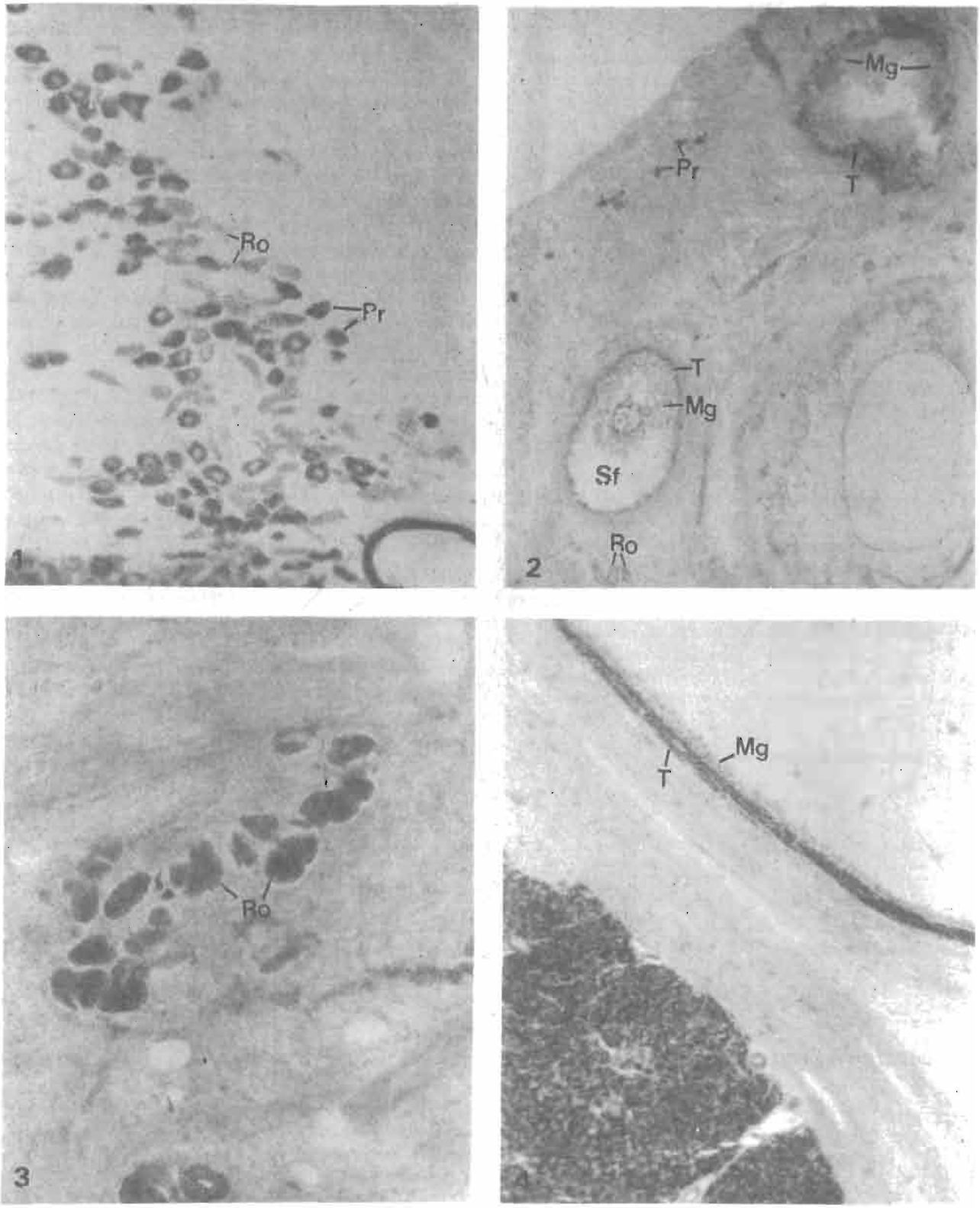
The study was conducted on the ovaries of 46 (neonatal 13, prepubertal 3, cyclic 22 and pregnant 8) normal healthy Murrah buffaloes. The fresh unfixed ovarian tissues were collected in liquid nitrogen for histoenzymic demonstration of NADH-diaphorase and NADH-diaphorase. The tissues were put to cryostat microtome at -20°C to obtain sections at10 μm on clean glass slides and were incubated for NADH-diaphorase and NADPH-diaphorase (Pearse 1972).

RESULTS AND DISCUSSION

The NADH-diaphorase showed negligible to weak activity in the surface epithelium, tunica albuginea and ovarian stroma in the buffalo ovary (Fig.2) The primordial and primary follicles showed strong NADH-diaphorase activity in all age groups studied except cyclic where it was intense. The NADH-diaphorase was strong to intense in the theca cells of secondary and tertiary follicles, whereas a variable activity from weak to strong could be observed in the atretic follicles in all age groups (Fig.2). It indicated the decreasing trend of the NADH-diaphorase in the atretic follicles. It was moderate to strong in the membrana granulosa of growing follicles and reduced to weak to moderate in atretic follicles (Table 1). Guraya (1979) described those inner layers of the granulosa in the preantral follicles contained relatively more activity of NADH-diaphorase than their outer layer. The enzyme is related with steroidogenic activity. The NADH-diaphorase activity was weak in the zona pellucida and oocyte. The interstitial gland cells had moderate to intense activity. In the blood vessels the activity was weak to moderate. In the rete ovarii it was moderate to strong. The luteal cells had moderate to strong NADH-diaphorase activity (Fig. 4). It supports the

*Part of Ph.D. Thesis.

Present address: [1]Associate Professor, HPKVV, Palampur 176 062. [2]Professor and Head, Department of Anatomy and Histology.



Figs 1- 4. **1**. Distribution of NADPD-diaphorase activity in the primordial follicles (Pr) and rete ovarii (RO of pregnant buffalo. Nitro BT method × 70. **2.** NADH-diaphorase activity in the primordial follicle (Pr), membrane granulosa (Mg), theca cells (T) of secondary follicle (SF) and atretic follicle (AF). The rete ovarii (RO) also showing activity. Nitro BT method × 18. **3**. Distribution of NADPH- diaphorase activity in the rete ovarii (RO). Nitro BT method × 70. **4**. Distribution of NADH-diaphorase activity in the membrana granulosa (Mg) and theca (T) of the tertiary follicle. The luteal cells are also positive. Nitro BT method × 70.

					Follicle									Cortex				Medulla						
					Secondary				Graafian tertiary					Atretic										
	Surface epithelium	Tunica albuginea	Cortical stroma	Primordial and primary	Theca	Membrane granulosa	Zona pellucida	Oocyte	Theca	Membrane granulosa	Zona pellucida	Oocyte	Theca	Membrane granulosa	Zona pellucida	Oocyte	Interstitial gland cells	Medullary strotma	Blood vessels	Neres ganglion	Rete ovarii	Hilar glands	Corpus luteum
Neonatal																							
NADH diaphorase	+	+	+	+++	+++	+++	+	+	+++	++	+	+	+++	+	+	+	+++/++++	+	++	0	+++	-	-
NADPH diaphorase	+	+	+	+++	+++	++	+	+	+++	++	+	+	+++	+	+	+	+++	+	++	0	+++	-	-
Prepubertal																							
NADH diaphorase	+	0/+	+	+++	+++	+++	0	+	+++/++++	++	0	+	+	+	0	+	++	+	+	-	++	-	-
NADPH diaphorase	+	0/+	0/+	+++	+++	+	0	+	+++	+	0	+	0/+	0/+	0/+	+	++	0/+	+	-	+	-	-
Cyclic																							
NADH diaphorase	+	0/+	+	++++	+++	++	0	+	+++/++++	++	0	+	0	0	-	-	++	+	++	++	+++	++	+++
NADPH diaphorase	+	+	+	++++	+++	++	-	+	++++	++	-	+	++/+++	+	-	+	+++	+	+	-	++	-	++/+++
Pregnant																							
NADH diaphorase	+	+	+	+	+++	++	-	-	+++	++	-	-	+++	+	-	-	++	+	+/	-/++	++	-	-/
NADPH diaphorase	+	+	+	+	+++	++	-	-	+++	++	-	-	+++	0/+	-	-	=	+	+	-	++	-	+++/++++

-, Not observed; 0, negative; 0/+, negligible; +, weak; ++, moderate; +++, strong; ++++, intense.

earlier findings of Singh (1994) in buffalo. The enzyme related with steroidogenic activities in the luteal cells.

The NADH-diaphorase activity was negligible to weak in surface epithelium, tunica albuginea and ovarian stroma. The NADPH-diaphorase activity in the primordial and primary follicles was strong to intense in all age groups of buffalo studied with the exception that in pregnant group it was weak (Fig.1). The activity in the theca was strong to intense in all the follicles. Negligible activity was observed only in the theca cells of atretic follicles of the prepubertal group. In the membrane granulosa the activity was moderate in secondary and tertiary follicles. It reduced to negligible and raised to moderate in the membrane granulosa cells of atretic follicles (Table 1). Roy and Saigal (1985) reported weak activity in the theca cells and moderate activity in the membrane granulosa cells of the sheep ovary. The enzyme participated in the steroidogenises by converting cholesterol to progesterone (Sorenson and Singh 1973). The NADPH-diaphorase activity was weak in the zona pellucida and oocyte. The interstitial gland cells of the ovary showed moderate to strong activity for NADPH-diaphorase confirmed the earlier findings of Roy and Saigal (1985) in sheep. The activity in blood vessels was weak to moderate and moderate to strong in the rete ovarii (Fig. 3). Roy and Saigal (1985) reported weak activity in the blood vessels of the sheep ovary. There was moderate to strong activity in the luteal cells as reported in sheep (Roy and Saigal 1985) and buffalo (Singh 1994). Sorenson and Singh (1973) reported that NADH-diaphorase was involved in conversion of cholesterol to progesterone in the corpus luteum..

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Histomorphology of the Harderian gland in White Leghorn birds*

ASHOK PAWAR[1], A VENKATKRISHNAN[2], C VIJAYARAGAVAN[3] and V RAMKRISHNA[4]

Tamil Nadu Veterinary and Animal University, Chennai, Tamil Nadu 600 007 India

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ABSTRACT

The Harderian gland of the White Leghorn bird is a compound tabulo-acinar gland surrounded by a thin connective tissue capsule. The septa divided the gland into lobules of varying size and the parenchyma of the gland contained acini at the periphery and varying degree of tubules towards the centre. The acini and tubules were lined by columnar cells on distinct basement membrane. Myoepithelial cells were observed. The interstitial tissue contained lymphocytes, fibroblasts, erythrocytes, myoid cells, mast cells and plasma cells. The electron micrographs revealed plasma cells possessing large number of dilated rough endoplasmic reticulum and secretory cells contained large number of moderate electron dense secretory vesicle with an electron-lucent core and golgi bodies.

Key words: Birds, Histomorphology, Harderian gland

The presence of the Harderian gland was first reported in 1694 by Johann Jacob Harder in the deer and suggested its role to moisten the surface of the eye. Light microscopic and ultrastructure study has been made in duck (Wight and Mackenzie 1974) and turkey (Maxwell *et al.* 1986) respectively. But, no attempt has been made in the domestic White Leghorn birds. Hence, the present study has been under taken to study its histomorphology and ultrastructure of the Harderian gland in White Leghorn birds.

MATERIALS AND METHODS

The Harderian gland tissue from the ventromedial aspect of the eye ball were collected from 42 White Leghorn birds aged from 1 to 62 weeks with an interval of 3 to 6 weeks from poultry research station, Nandanam, Chennai, for histomorphological study. The tissue pieces were fixed in fixatives like buffered neutral formalin, Bouin's fluid susa's fluid and zenker's fluid. The fixed tissues were processed and embedded in paraffin. The sections of 6-8μ were cut and stained by Mayer's haemalum-eosin-phloxin method for general observation, Azan staining method for connective tissue, Verhoeff's elastic stain method for elastic fibres (Singh and Sulochana 1978), Gomori's methods for reticulin, Mallory's phosphotungstic acid haematoxylin method (PTAH) for muscle and collagen fibres (Luna 1968) and Silver impregnation method for reticular fibres (Humason 1979).

For ultrastructure study, the tissue pieces were fixed immediately after collection, in 2.5% glutaraldehyde phosphate buffer for 3 hr at 4°C and washed thoroughly in phosphate buffer and post fixed in 1% osmium tetraoxide at 4°C for 1 hr. The pieces were dehydrated in ascending grades of acetone and infiltrated in 2 steps with Epon mixture and propylene oxide in the proportion of 1:1, 2:1 and finally in Epon mixture alone. The tissues were embedded in beem capsule and polymerised at 60°C for 72 hr. The ultrastructure section of 600-900 A were cut. These sections were stained with uranyl acetate and lead citrate and examined under Jeol electron microscope (Hayat 1972).

RESULTS AND DISCUSSION

Stroma

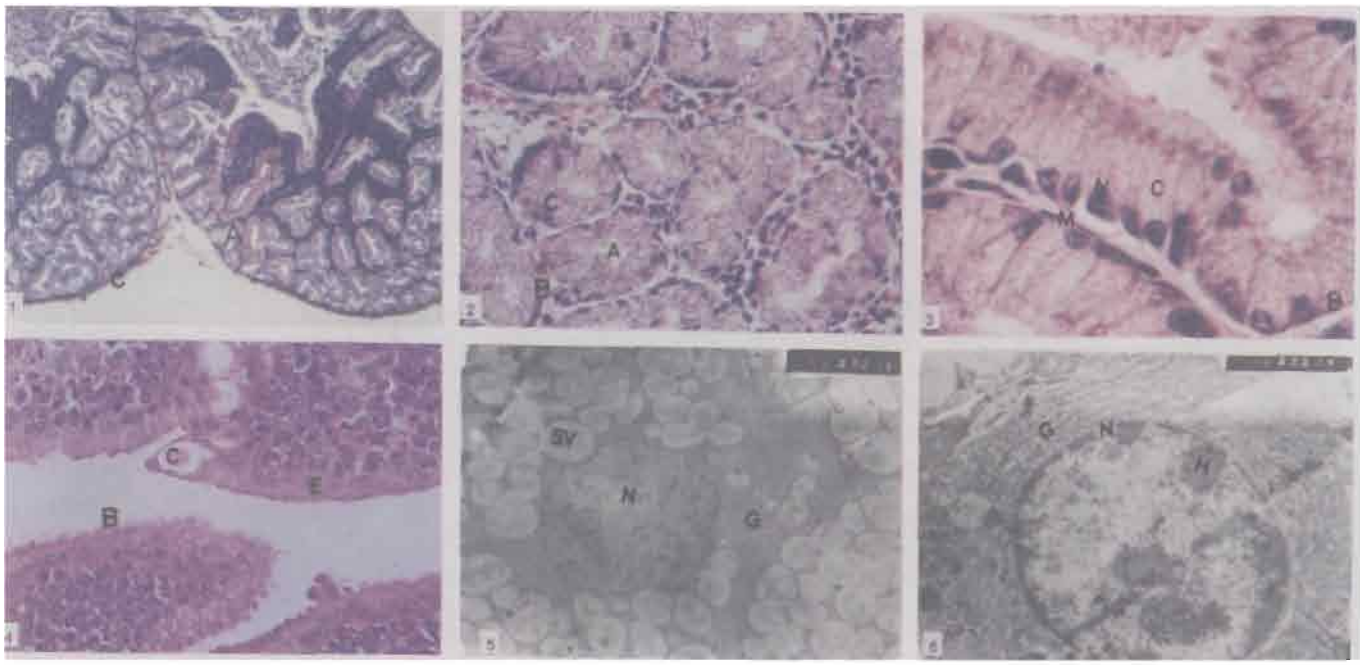
The Harderian gland of White Leghorn bird was a compound tubulo-acinar type with a thin connective tissue capsule which was composed of collagen, elastic and reticular fibres. From the capsule the connective tissue trabeculae penetrated into the gland and divided the gland into lobules of varying sizes. In addition to its fibrous components the capsule possess nerve fibres, autonomic ganglia and different order of blood vessels (Fig. 1). This finding was in agreement with the earlier reports of Wight *et al.* (1971) and Maxwell *et*

*Part of the M.V.Sc. Thesis of the first author submitted in the Tamil Nadu Veterinary and Animal Sciences University, Chennai 600 007.

Present address: [1]Assistant Professor, [4]Professor and Head, Department of Anatomy and Histology, Veterinary College, Bidar. Karnataka 585 401.

[2]Formerly Professor, 101, Lake View Road, West Mambalam, Chennai 600 033.

[3]Professor and Head, Department of Anatomy, Madras Veterinary College, Chennai 600 007.



Figs 1-6. **1.** Photomicrograph of the Harderian gland of 30 week-old White Leghorn bird showing a panoramic view of the gland. H & E ×80. C - Capsule, A - acini, T - tubules, I - interstitial tissue. **2.** Photomicrograph of the Harderian gland of 20 week-old White Leghorn bird showing columnar cells in the acini. H & E ×500. A - acini, C - columnar cell, B - basement membrane, N - nucleus, Cap - inter acinar capillary. **3.** Photomicrograph of the Harderian gland of 20 week-old White Leghorn bird showing columnar cells in the tuble. H & E×1250. C - columnar cell, N - nucleus, B - basement membrane, M - myoepithelial cell. **4.** Photomicrograph of the Harderian gland of 35 week-old White Leghorn bird showing blebs at luminal surface of epithelium. H & E×500. E - Ephithelium, C - cyst, I - interstitial tissue, B - blebs. **5.** Electron micrograph of the Harderian gland of White Leghorn bird showing vesicle in the secretory cell. Uranyal acetate ×6600. SV - Secretory vesicle, N - nucleus, G - Golgi body. **6.** Electron micrograph of the Harderian gland of White Leghorn bird showing plasma cell. Uranyal acetate×13000. G - Granular endoplasmic reticulum, N - nuclear membrane, H - heterchromatin.

al. (1986) in domestic fowl and turkey respectively. But differed with the report of Burns (1992) where the gland was compound tubular in penguins.

Parenchyma

The parenchyma of the Harderian gland in White Leghorn bird was consisted of acini at the periphery and tubules towards the centre with interstitial tissue between them (Fig. 1). The acini and tubules in the parenchyma of the Harderian gland were lined by columnar cells with basal spherical nuclei. A distinct basement membrane could be observed in the acini and tubules which were surrounded by rich network of capillaries (Figs 2, 3). The type of the epithelium was similar, to the earlier report of Wight *et al.*(1971) in domestic fowl and Maxwell *et al.* (1986) in turkey.

The myoepithelial cells, surrounded the acini and tubules were present inside the basement membrane with their process extended in the interstices of the lining cells of acini and tubules (Fig. 3). Existence of myoepithelial cells in the Harderian gland associated with acini and tubules has also been reported by Rothwell *et al.* (1972) in fowl, Weaker (1981) in armedllo and Abou Elmagard (1992) in camel.

The tubules of the Harderian gland were lined by columnar cells and the occurrence of myoepithelial lymphocytes was common in the lining epithelium. Del cacho *et al.* (1992) stated that the intraepithelial lymphocytes might be involved in antigen transportation.

Cyst formation was very frequent in the lining epithelium of tubules of the adult bird. The cellular population in the interstitial tissue constituted lymphocytes macrophages, mast cells, fibroblast, erythrocytes, myoid cells and plasma cells (Fig.4). The cellular population particularly the plasma cells increased with age which corroborated the finding of Bang and Bang (1968) in fowls, pheasants, turkey and pigeons.

The lining of columnar cells of the tubules contained secretary blebs on the luminal surface. The blebs were found more in number in the main collecting tubules indicating apocrine secretion (Fig.4). This finding was in agreement with the report of Bjorkman *et al.* (1960) in rabbit.

Electron micrograph of the gland revealed, the presence of large number of moderated electron dense secretory vesicles with an electron lucent core with golgi bodies (Fig.5). The plasma cell was possessing a large nucleus containing heterochromatin in clumps adjacent to the nuclear membrane. Dilated profile of the granular endoplasmic reticulum contained moderately dense homogenous material. (Fig.6). This finding resembles with the finding of Rothewell *et al.* (1972) in domestic fowl and Maxwell *et al.* (1986) in turkey.

It was concluded that Harderian gland of the White Leghorn birds was compound tubulo-acinar type surrounded

by thin capsule. The acini and tubules were lined by columnar cells and interstitial tissue contained large number of cell population mainly lymphocytes and plasma cells indicating its role in immunity. The mode of secretion was by apocrine mechanism.

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Effect of concurrent hypophosphorosis and molybdenosis on some mineral profile in buffalo calves (*Bubalus bubalis*)

ASHWANI KUMAR[1], S S RANDHAWA[2] and RAJVIR SINGH[3]

Punjab Agricultural University, Ludhiana, Punjab 141 004 India

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ABSTRACT

Buffalo calves were fed phosphorus deficient diet for 90 days, and in 1 group simultaneous feeding of molybdenum @ 3 mg/kg body weight was also done to investigate the effect of concentration of molybdenum, phosphorus, iron and copper on different body fluids, viz. plasma, rumen liquor and cerebrospinal fluid (CSF) at different time intervals. Significant decline in plasma inorganic phosphorus was observed on day 45 in group T_2 (fed on phosphorus deficient and molybdenum supplemented diet) and group T_3 (fed on phosphorus deficient diet alone) as compared to group T_1 (healthy control). Animals of group T_2 showed more gradual declining trend accompanied by intense fall on day 90. Plasma inorganic phosphorus decreased on day 45, which was followed by fall in rumen liquor phosphorus on day 60 in both the groups. Nonsignificant difference was noted in groups T_2 and T_3 regarding CSF and rumen liquor phosphorus concentration. In group T_2 plasma molybdenum increased significantly and gradually after day 15 following feeding of molybdenum, whereas, plasma copper declined significantly on day 45. In molybdenum supplemented animals copper concentration decreased initially (day 30) in rumen liquor followed by fall in plasma on day 45 and in CSF on day 90. Iron concentration in all the groups in different body fluids showed inconsistent and nonsignificant fluctuation.

Key words: Buffalo, Calves, Cerebrospinal fluid, Copper, Hypophosphorosis, Interaction, Iron, Molybdenosis, Molydenum, Phosphorus, Plasma, Rumen liquor

Phosphorus (P) deficiency is widely prevalent in grazing livestock (McDowell 1992). It occurs either due to lower intake or its antagonistic interaction with various other minerals like calcium (Ca), molybdenum (Mo), sulphur (S), aluminium (Al) and iron (Fe) etc. in soil, plant, rumen liquor, blood and various body fluids. The antagonistic interaction of phosphorus and molybdenum assume more importance because of incrimination of both minerals with various conditions like poor growth rate, weight loss, bone disorders accompanying lameness, haemoglobinuria, rough hair coat, decreased production and reproductive disorders in dairy animals. Moreover, in a recent survey in 4 central districts of Punjab, hypophosphataemia was recorded in 1/3rd of the dairy animals, whereas, 40% of the animals had higher levels of molybdenum in their blood (Randhawa 1999). The present experiment was therefore conducted to investigate the effect of experimentally induced phosphorus deficiency alone and in combination with molybdenum supplementation on the status of various relevant/antagonistic elements, viz. phosphorus, molybdenum, iron, copper in plasma, rumen liquor (RL) and cerebrospinal fluid (CSF) in buffalo calves.

Present address: [1]Assistant Professor, [2]Professor-cum-Head, Department of Clinical Veterinary Medicine, Ethics and Jurisprudence; [3]Professor-cum-Head, Department of Veterinary Physiology.

MATERIALS AND METHODS

Male buffalo calves (20), 1-year old and weighing between 68 and 106 kg with average body weight of 89 kg, were used for the present study. They were dewormed with broad spectrum anthelmintic, freed from external parasites and acclimatized for a month under identical management conditions prior to start of study. The cows were randomly divided into 3 groups T_1, T_2 and T_3 where group T_1 consisted of 5 animals and served as healthy control, T_2 of 10 animals and T_3 of 5 animals.

Phosphorus deficiency was induced in groups T_2 and T_3 by feeding phosphorus deficient diet for 90 days. The phosphorus deficient diet consisted of urea treated wheat straw and phosphorus deficient mineral mixture along with supplementation of vitamins A and D @ 150 iu/kg of mineral mixture. The calves of group T_2 were also given orally 10% ammonium molybdate solution in drinking water to provide molybdenum @ 3 mg/kg body weight. To establish control values (0 day), rumen liquor, blood and CSF samples were

collected simultaneously from each animal on 2 occasions at 2 days interval. Heparinised blood samples from all the animals were collected at 15 days interval for 90 days throughout the period of study and the plasma separated from blood was stored in 5 ml acid washed plastic storage vial at –15°C until used for various mineral estimations. The rumen liquor samples were collected from each animal before feeding with the help of stomach tube in the morning at 30 days interval throughout the study. Rumen liquor was strained through double-layer of muslin cloth and stored at –15°C for estimation of various minerals. Similarly, cerebrospinal fluid from each animal of groups T_2 and T_3 was collected by lumbosacral tap at 30 days interval and stored at –15°C. The inorganic phosphorus was estimated spectrophotometrically and molybdenum was estimated spectrophotometrically by acetone reduction of thiocyanate (Ellis and Olson 1950). For estimating concentrations of copper and iron a known volume of sample was digested with triple acid (concentrate nitric acid + 70% perchloric acid + concentrate sulphuric acid) in the ratio of 10: 3: 1. The final volume was made up to 8 ml with triple distilled water and the samples were run through atomic absorption spectrophotometer (Ludmilla 1976). The results were subjected to student's 't' tests. Significance of difference of the mean values of various parameters between groups T_1,T_2 and T_1 and T_3 was tested at 1 and 5% level of probability by applying simple t-test (Snedecor and Cochran 1976).

RESULTS AND DISCUSSION

The alterations in concentration of different elements in plasma, rumen liquor and CSF of all the 3 groups are presented in Tables 1, 2 and 3 respectively. The mean plasma inorganic phosphorus (Pi) in groups T_2 and T_3 after 45th day was significantly ($P<0.01$) lower as compared to healthy control and were also well below the normal range (6-8 mg/dl) in young calves and adults (4-6 mg/dl) given by Underwood (1981). There are divergent views about indication of plasma inorganic phosphorus levels as a dietary status in cattle. McDowell (1992) stated that first response to a dietary deficiency of phosphorus was a fall in the inorganic phosphate fraction of blood plasma and withdrawal of its reserves in the bones. However, studies of Cohen (1973) revealed that blood level of inorganic phosphorus was maintained for long period despite severe deficiency of element indicating that blood phosphorus was not the good indicator of phosphorus status in cattle. The significant fall of plasma phosphorus occurred on day 45, and it persisted at a lower level throughout the study. Haque and Verma (1990) reported significant decline in levels of plasma inorganic phosphorus on 65th day (4.4 ±

Table 1. Concentration of minerals in plasma of experimental buffalo calves

Parameters	Group	Days						
		0	*15*	*30*	*45*	*60*	*75*	*90*
Inorganic phosphorus (mg/dl)	T_1	7.03 ±1.06	7.77 ±1.21	6.23 ±0.81	6.03 ±0.94	6.54 ±1.19	6.97 ±0.81	6.72 ±0.77
	T_2	6.50 ±1.06	6.03 ±0.83	5.81 ±1.18	4.73** ±1.27	4.03** ±0.49	3.03** ±0.63	2.60** ±0.47
	T_3	6.15 ±1.13	5.95 ±1.17	4.78 ±1.27	3.41* ±0.78	2.95** ±0.63	3.50** ±0.49	2.72** ±0.49
Copper (µg/dl)	T_1	111.57 ±15.38	105.2 ±15.01	109.31 ±7.34	127.26 ±6.42	127.90 ±12.84	116.31 ±6.56	113.14 ±8.53
	T_2	168.78 ±40.70	166.60 ±26.25	142.15 ±107.70	106.60** ±10.00	109.00** ±10.38	93.25** ±10.01	96.72** ±8.20
	T_3	115.48 ±15.38	111.03 ±7.67	106.60 ±0.00	119.90 ±13.32	118.00 ±60.10	114.34 ±20.52	110.47 ±20.22
Iron (µg/dl)	T_1	417.16 ±20.30	338.85 ±18.36	352.54 ±8.51	413.24 ±21.62	542.10 ±40.68	606.50 ±18.80	616.00 ±17.73
	T_2	377.68 ±104.04	302.14 ±67.04	533.22 ±21.52	423.22 ±17.48	564.30 ±18.70	624.30 ±16.58	619.17 ±14.23
	T_3	357.72 ±62.34	319.92 ±51.04	513.30 ±18.94	406.54 ±34.62	595.42 ±10.77	612.55 ±13.92	618.30 ±16.53
Molybdenum (µg/ml)	T_1	0.93 ±0.21	0.98 ±0.11	1.07 ±0.18	-	1.13 ±0.13	1.25 ±0.27	1.17 ±0.16
	T_2	1.06 ±0.16	2.11* ±0.81	3.71** ±1.78	-	6.22** ±0.83	8.15** ±0.91	9.11** ±1.02
	T_3	0.87 ±0.23	0.99 ±0.31	-	1.09 ±0.20	-	1.17 ±0.39	1.20 ±0.36

** Significant at 1% level; * significant at 5% level.

0 41 mg%) as compared to zero day (8.8 ± 0.5 mg%) in crossbred dairy calves fed on phosphorus deficient diet, whereas, Blair-West *et al.* (1992) recorded fall in inorganic fraction of plasma phosphorus to 0.86 ± 0.04 mM in 6 months study on adult cattle in induced phosphorus deficiency with parotid saliva drainage and phosphorus deficient diet.

Declining trend in inorganic phosphorus in groups T_2 and T_3 was more consistent and was more gradual in group T_2 animals as compared to those in group T_3 (Table 1). The average zero day phosphorus of group T_2 animals at the start of study was slightly more than group T_3 animals, which fell below group T_3 at 90th day. This more marked decrease in plasma phosphorus in group T_2 as compared to group T_3, however, insignificant statistically might be due to more excretion of phosphorus through urine and faeces (Clarke and Clarke 1967) due to gradual mobilization of phosphorus from skeletal reservoirs because of feeding of molybdenum (Shirley *et al.* 1950, Clarke and Clarke 1967).

There was significant decrease (Table 2) in concentration of phosphorus in rumen liquor of groups T_2 and T_3 animals as compared to control animals after day 60. This could be ascribed to feeding of phosphorus deficient diet. Rumen liquor phosphorus concentration represent dietary phosphorus and phosphorus coming through saliva to rumen liquor as there is no absorption of phosphorus from rumen and is mainly absorbed from small intestine (Thomson *et al.* 1964). Parotid saliva of ruminants as compared to other species contains 10-times more concentration of phosphorus than that of plasma (Compton *et al.* 1980, Wright *et al.* 1984), and its

Table 2. Mineral concentration in rumen liquor of experimental buffalo calves

Parameters	Group	Days			
		0	30	60	90
Inorganic phosphorus (mg/dl)	T_1	15.92 ±1.40	17.82 ±2.92	18.00 ±1.65	15.41 ±1.27
	T_2	16.53 ±1.81	16.93 ±0.83	12.80** ±0.62	13.20** ±0.47
	T_3	16.13 ±3.28	15.73 ±3.19	12.66** ±2.68	13.40** ±0.31
Copper (µg/dl)	T_1	160.34 ±19.50	159.90 ±0.00	186.55 ±30.77	151.00 ±30.70
	T_2	168.76 ±12.54	1115.40** ±40.69	159.90** ±67.00	106.06** ±0.00
	T_3	151.00 ±55.48	124.40** ±30.76	177.60 ±39.75	159.90 ±15.20
Iron (µg/dl)	T_1	2619.15 ±547.76	2412.80 ±502.80	2825.90 ±673.21	3199.00 ±1071.76
	T_2	2132.80 ±832.16	2532.80 ±673.20	4865.50** ±659.73	5199.00** ±689.23
	T_3	3065.90 ±757.05	2799.30 ±733.03	3599.20* ±188.37	2932.60 ±532.70

**Significant at 1% level; *significant at 5% level.

concentration may fall with fall in plasma phosphorus. In this study, the concentration of plasma inorganic phosphorus fell significantly on 45th day that might have led to decreased salivary phosphorus concentration, which when coupled with decreased phosphorus intake resulted into fall in rumen liquor phosphorus concentration in both the groups at 60th day as compared to respective control. Blair-West *et al.* (1992) also experimentally revealed that phosphorus of parotid saliva also decreased in hypophosphataemia associated with feeding of phosphorus deficient diet, and this reduction in salivary phosphorus was associated with a fall in the phosphorus of ruminal fluid from 15 mmol/l to 5 mmol/l after 6 months in adult cattle. Rodheuscord *et al.* (1994) observed that phosphorus concentration in rumen liquor started to fall immediately with changing phosphorus intake. They observed a minimum value of 0.28 mM in rumen liquor and noted similar trend in plasma also. From the nonsignificant differences in concentration of phosphorus in plasma and rumen liquor noted in both the groups T_2 and T_3, it could be inferred that molybdenum did not interfere with the absorption of phosphorus at intestinal level. There was gradual decrease in CSF phosphorus contents with decrease in phosphorus in blood and was contrary to the findings of Blair-West *et al.* (1992) who reported no change in concentration of CSF phosphorus contents. This could be due to species difference or difference in the age of animals studied in both the experiments. The parallel decline in the CSF phosphorus could be correlated with the decreased synthesis of ATP (Lichtman *et al.* 1971) because of phosphorus deficiency thus resulting into less active transport across blood-brain barrier (Guyton 1986).

Plasma copper concentration at the beginning of experiment ranged from 111 to 168 µg/dl and thus were in the normal to higher range of normality as stated by Dewes *et al.* (1990) in mammalian species (9.60 – over 15.99 µmol/l). Plasma copper content showed nonsignificant alterations (Table 1) up to 45th day in phosphorus deficient and molybdenum supplemented calves. This could be due to mobilization of copper from hepatic stores because of oral supplementation of molybdenum (Kincaid 1980). Miller (1974) also stated that tissue deposition and withdrawal were key routes in copper homeostasis. Plasma concentration of copper decreased significantly (Table 1) in molybdenum supplemented calves at 45th day which remained consistently low up to 90th day of experimentation. Various workers reported different fluctuation in plasma copper concentration with molybdenum supplementation at variable time intervals in different species; Wang *et al.* (1988) reported no change in plasma copper by feeding molybdenum (35 ppm DM) and sulphur (0.3%) in Jersey calves in 24 weeks; Randhawa (1999) by feeding 30 ppm of molybdenum in diet for 6 months in buffalo calves observed no significant change in plasma copper. In hypophosphataemic animals of group T_3 plasma copper was relatively constant and remained comparable to

Table 3. Mineral concentrations in CSF of experimental buffalo calves

Parameters	Group	Days			
		0	30	60	90
Inorganic phosphorus (mg/dl)	T_2	0.98 ±0.13	1.00 ±0.14	0.56** ±0.11	0.21** ±0.17
	T_3	1.46 ±0.40	1.41 ±0.96	0.59** ±0.18	0.40** ±0.29
Copper (µg/dl)	T_2	112.40 ±20.32	106.50 ±18.03	106.50 ±19.23	88.75* ±15.97
	T_3	106.50 ±19.17	100.50 ±17.06	71.00 ±12.78	75.45 ±13.58
Iron (µg/dl)	T_2	2400.20 ±432.00	2489.00 ±145.33	2222.50* ±400.05	2266.80 ±44.25
	T_3	2248.30 ±209.91	2311.00 ±416.03	2133.60 ±384.04	2103.80 ±357.90

**Significant at 1% level; *significant at 5% level.

control group. Concentration of copper in rumen liquor in group T_2 was significantly lower from 30th day onwards (Table 2). This could be due to known formation of copper thiomolybdate complexes in the rumen (Radostitis *et al.* 1994) which were more readily absorbed than their oxygen analog molybdate (Hidiroglou *et al.* 1990). Copper concentration of CSF declined (Table 3) in both T_2 and T_3 groups, which was more significantly marked in group T_2 at 90th day and was ascribed to molybdenum ingestion. Howel *et al.* (1964) also recorded decline in copper contents in brain, whereas, Ivan *et al.* (1990) reported decrease in copper contents in spinal cord in molybdenum fed animals. As per trend of copper in plasma, rumen liquor and cerebrospinal fluid given in Tables 1, 2 and 3 it could be inferred that copper concentration in CSF was last to fall preceded by fall in rumen liquor and plasma copper concentration. Plasma iron concentration (Table 1) in healthy buffalo calves was more than the normal range (89-253 µg/dl) given by Underwood (1981) in healthy cows. The values were, however, comparable to the values given by Patel (1969) in Surti buffaloes. The phosphorus deficiency and molybdenum supplementation had no impact on plasma iron concentration as revealed by values at different times of sampling in groups T_2 and T_3 as compared to healthy animals. Rumen liquor, iron concentration showed a wide range of 2412.80 ± 502.80 to 3199.00 ± 1071.76 µg/dl in healthy controls. There was significant increase in iron concentration in rumen liquor from 60th day onward in group T_2 which could be due to feeding of molybdenum which can interfere with iron absorption by competing for absorption binding sites in the gut, however, the corresponding decline in plasma iron was not observed. The CSF iron concentration showed insignificant changes in both the groups as compared to zero values with a single significant deviated value at 60th day in group T_2.

The mean plasma molybdenum concentration in control animals varied nonsignificantly during the experiment. A similar trend was observed in phosphorus deficient animals of group T_3. The control values were higher than the control mean plasma molybdenum concentration reported by Randhawa (1999) in buffalo calves (0.00 – 0.36 ppm) and by Humphries *et al.* (1983) in cow calves (0.01 – 0.02 mg/litre). Buffalo calves on molybdenum supplemented and phosphorus deficient diet recorded a gradual and significant increase (Table 1) in plasma molybdenum by the end of trial on 90th day. These results are similar to the earlier findings in cattle (Lesperance 1985) and buffaloes (Randhawa 1993) where significant increase was observed by 30th day, however, disagreement with Randhawa (1999) in buffalo calves who recorded increase in plasma molybdenum from 0.37 – 2.82 ppm after 180 days by feeding diet containing 30 ppm of molybdenum. The difference could be due to accompanying deficiency of phosphorus that was present in this trial.

On the basis of the results of present study it could be inferred that molybdenum intake aggravates the phosphorus deficiency.

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Profile of some marker enzymes during endotoxic shock in buffalo calves supplemented with vitamin E and selenium

NEERAJ SHARMA[1], SHASHI NAYYAR[2] and S P S SINGHA[3]

Punjab Agricultural University, Ludhiana, Punjab 141 004 India

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ABSTRACT

A study was undertaken to evaluate the profile (1-72 hr post infusion) of some marker enzymes during endotoxic shock in buffalo calves, and to assess the role of prophylactically supplemented vitamin E and selenium in alleviating the endotoxic effect. Buffalo calves (15), 6-8 months old were divided into 3 groups. Group 1 (control) - infused with 0.9% normal saline; group 2 – infused with *E. coli* endotoxin @ 5 µg/kg body weight in normal saline solution; group 3 – supplemented prophylactically with 250 mg vitamin E and 7.5 mg selenium injections at weekly intervals for 1 month prior to induction of endotoxic shock. Endotoxin caused significant increase in the activities of creatine kinase, lactate dehydrogenase, sorbitol dehydrogenase and arginase. These changes were less pronounced in vitamin E-selenium supplemented calves. Acetyl choline esterase and monoamine oxidase activities decreased significantly with endotoxic shock at 1 hr post infusion with no effect of vitamin E and selenium being seen.

Key words: Buffalo, Calves, Endotoxic shock, Enzymes, Selenium, Vitamin E

Endotoxic shock is quite common in veterinary practice as the gastro-intestinal tract of ruminants is a store house of gram negative bacteria and potential pool of endotoxin. Cattle and buffalo are extremely sensitive to the effects of endotoxin (Constable *et al.* 1993). The reactive oxygen species are important mediators of cellular damage during endotoxic shock (Portoles *et al.* 1996). The severe oxidative stress due to production of free radicals in endotoxic shock, if not controlled, can cause extensive cell damage leading to the release of various tissue specific enzymes into systemic circulation because of alterations in cell membrane permeability and cell necrosis. Vitamin E and selenium have a direct role in protection of cell membranes from free radicals. Keeping in view the immense antioxidant role of vitamin E and selenium the activities of enzymes sorbitol dehydrogenase, creatine kinase, lactate dehydrogenase, arginase, monoamine oxidase and acetyl choline esterase were determined before and after induction of endotoxic shock in buffalo calves with/without prophylactic supplementation of vitamin E and selenium.

MATERIALS AND METHODS

Buffalo calves (15), 6-8 months old, were maintained as per the standard management and feeding conditions practised at the Punjab Agricultural University, Ludhiana. The buffalo calves were divided into 3 groups of 5 each:

Group 1 (control) – This group was administered 100 ml normal saline solution intravenously over half-an hour.

Group 2 (endotoxic shock) - The animals were infused with *E.coli* endotoxin (lyophilized phenol extracted *E.coli* endotoxin 0111: B4 lipopolysaccharide) @ 5 µg/kg body weight, dissolved in 100 ml normal saline solution by a slow intravenous injection over half-an hour.

Group 3 (vitamin E-selenium + endotoxic shock) – The animals in this group were supplemented with 250 mg vitamin E and 7.5 mg selenium in the form of i/m injections at weekly intervals for 1 month prior to induction of endotoxic shock.

The blood samples were collected at 0 hr (before the induction of shock), 1,3,6,9,12,24,48 and 72 hr after the induction of endotoxic shock in all the groups. Plasma was separated in a refrigerated centrifuge at 3 000 rpm for 15 min and stored at –20°C till analyzed. Creatine kinase (CK) activity was estimated by chemistry analyser using autopack kits. Lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), arginase, acetyl choline esterase (AchE) and monoamine oxidase (MAO) were determined in plasma as per Wootton (1964), Gerlich (1983), Mia and Koger (1978), Voss and Sachsse (1970) and Tabor and Tabor (1971) respectively. The data were analyzed statistically as per Snedecor

Present address: [1]M.V.Sc. Student, [2]Professor, Department of Veterinary Biochemistry. [3]Biochemist, Department of Veterinary Anatomy and Histology, College of Veterinary Science.

and Cochran (1976).

RESULTS AND DISCUSSION

Creatine kinase

The CK activity (Table 1) in group 2 increased significantly as compared to group 1 at 3 hr post-infusion, attained the peak value at 12 hr and then decreased till 72 hr post infusion, but did not return to the basal value in control group. In group 3, the CK activity increased significantly at 6 hr post infusion, attained its peak value at 12 hr post infusion and declined thereafter till 72 hr post infusion. The CK activity was significantly higher in both groups 2 and 3 as compared to group 1 at 72 hr. As compared to control group, the increase in CK activity was significantly lower in group 3 as compared to group 2.

The increased level of plasma CK after the induction of endotoxic shock indicated myopathy, as CK is an important enzyme of striated muscles and is useful in diagnosis of skeletal muscle damage. Rise in CK level is also indicative of deficiency of vitamin E and selenium (McMurray and Mc Eldowney 1977, Kennedy *et al.* 1987) and characterized by myonecrosis of skeletal and cardiac muscles. The supplementation of vitamin E and selenium in group 3 animals might have protected the skeletal and cardiac muscles against oxidative stress by inhibiting the formation of lipid peroxides, which is reflected in the lesser increase in CK activity of group 3 animals.

Lactate dehydrogenase

The LDH activity in group 2 (Table 2) showed a significant increase as compared to group 1 at 3 hr post infusion of *E. coli* endotoxin; the activity continued to increase and attained its peak value at 24 hr post infusion. Thereafter, the LDH activity declined till 72 hr post-infusion but the value was higher than the basal value in control group. In group 3, there was significant increase in LDH activity at 9 hr post-infusion, attaining a peak value at 24 hr post-infusion. Thereafter, the LDH activity decreased till 72 hr post-infusion but the level was significantly higher than the basal value in control group.

Lactate dehydrogenase is found mainly in myocardium, skeletal muscles and to some extent in kidney and liver. The elevated levels of LDH during endotoxemia may be attributed to extensive changes in all of the above mentioned organs, caused by anoxia primarily due to shock and stasis of circulation (Wright *et al.* 1981) and myodegenerative changes caused by endotoxic shock (Kaszubkiewicz *et al.* 1981). Vitamin E has a strong capacity to protect the myocardium against lipid peroxidation even in the presence of endotoxin

Table 1. Effect of endotoxic shock on plasma creatine kinase, lactate dehydrogenase and sorbitol dehydrogenase activities in buffalo calves with prophylactic vitamin E and selenium supplementation

Group	Time (hr)								
	0 (Normal)	1	3	6	9	12	24	48	72
Creatine kinase (U/L)									
Group 1	140.2 ±12.4^{a}	148.6 ±11.1^{a}	157.8 ±12.1^{a}	160.0 ±12.4^{a}	157.0 ±9.0^{a}	146.8 ±10.8^{a}	151.0 ±11.2^{a}	146.0 ±11.5^{a}	142.6 ±12.7^{a}
Group 2	156.6 ±21.3^{a}	188.2 ±24.1^{a}	289.6 ±14.6^{*b}	289.6 ±55.5^{*b}	422.8 ±34.0^{*b}	436.6 ±29.5^{*b}	402.6 ±40.4^{*b}	310.2 ±35.6^{*b}	253.4 ±31.1^{*b}
Group 3	137.8 ±18.8^{a}	141.8 ±18.0^{a}	182.6 ±24.7^{a}	245.4 ±29.5^{*c}	294.6 ±34.0^{*c}	328.4 ±37.0^{*c}	325.0 ±37.7^{*c}	280.6 ±47.8^{*b}	244.0 ±42.5^{*b}
Lactate dehydrogenase (IU/L)									
Group 1	206.2 ±8.9^{a}	206.3 ±9.2^{a}	210.4 ±8.9^{a}	212.8 ±9.0^{a}	200.0 ±9.0^{a}	215.0 ±8.9^{a}	209.0 ±8.7^{a}	216.0 ±9.0^{a}	208.0 ±9.0^{a}
Group 2	216.8 ±9.6^{a}	217.8 ±10.0^{a}	249.1 ±12.8^{*b}	328.8 ±12.4^{*b}	363.5 ±16.5^{*b}	400.2 ±15.6^{*b}	485.7 ±17.9^{*b}	402.8 ±16.0^{*b}	346.7 ±15.3^{*b}
Group 3	193.4 ±15.3^{a}	193.8 ±15.8^{a}	201.5 ±15.1^{a}	216.9 ±16.1^{a}	250.3 ±20.3^{*c}	303.5 ±15.4^{*c}	338.9 ±14.8^{*c}	335.9 ±15.4^{*c}	310.6 ±20.6^{*c}
Sorbitol dehydrogenase (U/L)									
Group 1	2.1 ±0.2^{a}	2.0 ±0.3^{a}	2.4 ±0.4^{a}	1.9 ±0.2^{a}	2.1 ±0.1^{a}	2.4 ±0.2^{a}	2.2 ±0.3^{a}	2.3 ±0.2^{a}	2.1 ±0.2^{a}
Group 2	2.3 ±0.3^{a}	2.6 ±0.3^{a}	2.8 ±0.3^{a}	6.6 ±1.6^{*b}	8.7 ±0.8^{*b}	13.6 ±2.2^{*b}	16.5 ±2.3^{*b}	19.2 ±0.6^{*b}	19.7 ±1.7^{*b}
Group 3	1.7 ±0.5^{a}	1.8 ±0.6^{a}	2.0 ±0.7^{a}	4.8 ±1.6^{*b}	6.5 ±1.2^{*b}	9.2 ±1.3^{*c}	12.1 ±1.9^{*c}	12.3 ±1.8^{*c}	12.6 ±2.0^{*c}

Group 1, control; group 2, endotoxic shock; group 3, vitamin E and selenium + endotoxic shock; values in the columns having same superscripts do not differ significantly from each other ($P< 0.05$); values in the rows having asterisk differ significantly from the normal values ($P<0.05$).

(Rojas *et al.* 1996). The antioxidant action of vitamin E and selenium is clearly reflected in the lesser increase in LDH activity of group 3 animals as compared to group 2.

Sorbitol dehydrogenase

The SDH activity (Table 1) in group 2 increased significantly as compared to group 1 at 6 hr post-infusion and kept on increasing till 72 hr post-infusion. In group 3, the SDH level was lower than group 2 during 12 –72 hr although it was greater than the values in group 1. In group 3 too, the SDH activity showed significant increase at 6 hr post-infusion and increased till 72 hr post-infusion.

The plasma SDH is an important liver-specific enzyme. The endotoxin significantly increases the oxidative damage to liver proteins in guinea pig liver (Cadenas *et al.* 1998). The increased levels of plasma SDH may be associated with hepatic dysfunction during the endotoxic shock. The protective antioxidant action of vitamin E and selenium is shown by the lesser increase in SDH activity of group 3 animals as compared to the increase in group 2.

Arginase

The arginase activity (Table 2) increased significantly in group 2 as compared to group 1 at 9 hr post-infusion with a further significant increase at 12 hr post-infusion. Thereafter, it showed no significant variation. In group 3 too, the arginase activity increased significantly at 9 hr post-infusion with a further increase at 12 hr post-infusion and no variation thereafter. However, the increase in arginase activity was lesser in group 3 as compared to group 1.

E.coli. endotoxin has a direct cytotoxic effect on hepatocytes during the endotoxic process (Portoles *et al.* 1993), resulting in the elevated levels of plasma arginase activity. The mild rise in the level of plasma arginase in group 3 animals may be associated with the hepato protective effect of vitamin E and selenium.

Acetylcholine esterase

As compared to group 1, the activity of AchE (Table 2) in group 2 and group 3 decreased significantly at 1 hr post-infusion; and then it started returning to the normal level reaching a value with no significant difference (with group 1) at 9 hr post-infusion. Further it remained more or less the same, till 72 hr post-infusion. No significant difference was observed between the trend in groups 2 and 3 during the endotoxic shock, the level of acetylcholine is increased at neuroeffector junction (Hardman and Limbard 1996). A significant decrease in AchE levels may be due to its increased utilization to metabolize the elevated level of acetyl choline during endotoxic shock. There was no significant difference observed between

Table 2. Effect of endotoxic shock on plasma arginase, acetyl cholinesterase and monoamine oxidase activities in buffalo calves with prophylactic vitamin E and selenium supplementation

Group	Time (hr)								
	0 (Normal)	1	3	6	9	12	24	48	72
Arginase (IU/L)									
Group 1	2.0 ±0.3^{a}	2.1 ±0.4^{a}	1.9 ±0.3^{a}	2.2 ±0.3^{a}	2.4 ±0.4^{a}	2.3 ±0.3^{a}	2.3 ±0.3^{a}	1.9 0.4^{a}	2.2 ±0.3^{a}
Group 2	2.4 ±0.8^{a}	2.7 ±0.7^{a}	3.0 ±0.7^{a}	5.2 ±1.6^{a}	9.1 ±1.7^{*b}	18.7 ±6.2^{*b}	20.6 ±5.2^{*b}	22.4 ±4.5^{*b}	22.7 ±4.3^{*b}
Group 3	2.0 ±0.3^{a}	1.8 ±0.3^{a}	2.3 ±0.3^{a}	4.0 ±0.3^{a}	5.9 ±0.9^{*b}	11.4 ±1.1^{*c}	13.2 ±1.2^{*c}	13.0 ±1.1^{*c}	13.3 ±1.3^{*c}
Acetyl cholinesterase (nmol acetyl choline hydrolysed/min/ml)									
Group 1	174.5 ±16.6^{a}	172.2 ±15.8^{a}	173.9 ±17.1^{a}	174.7 ±17.0^{a}	175.2 ±16.9^{a}	174.3 ±17.0^{a}	176.0 ±9.7^{a}	173.2 ±17.1^{a}	175.9 ±17.8^{a}
Group 2	184.6 ±12.6^{a}	121.4 ±4.3^{*b}	123.2 ±3.9^{*b}	140.9 ±7.6^{*a}	163.3 ±13.6^{a}	170.5 ±13.0^{a}	171.6 ±12.1^{a}	172.8 ±13.0^{a}	175.1 ±12.0^{a}
Group 3	183.7 ±10.8^{a}	128.6 ±8.5^{*b}	124.3 ±5.9^{*b}	147.0 ±5.5^{*a}	168.7 ±9.1^{a}	169.1 ±8.9^{a}	173.9 ±10.0^{a}	176.0 ±12.0^{a}	180.3 ±10.6^{a}
Monoamine oxidase (clinical units/0.6 ml)									
Group 1	17.0 ±2.0^{a}	16.9 ±2.1^{a}	16.9 ±1.9^{a}	17.2 ±2.0^{a}	16.9 ±2.0^{a}	17.1 ±2.1^{a}	17.5 ±1.9^{a}	17.0 ±2.0^{a}	16.5 ±1.8^{a}
Group 2	18.8. ±2.0^{a}	27.5 ±2.1^{*b}	22.8 ±2.3^{a}	19.8 ±2.5^{a}	19.2 ±2.4^{a}	19.3 ±2.4^{a}	19.3 ±2.3^{a}	19.0 ±2.4^{a}	18.9 ±2.5^{a}
Group 3	17.5 ±1.9^{a}	25.4 ±2.0^{*b}	20.0 ±2.2^{a}	19.5 ±2.2^{a}	20.5 ±2.4^{a}	19.5 ±2.2^{a}	20.0 ±2.3^{a}	19.0 ±2.1^{a}	21.0 ±2.1^{a}

Group 1, control; group 2, endotoxic shock; group 3, vitamin E and selenium + endotoxic shock; values in the columns having same superscripts do not differ significantly from each other ($P< 0.05$); values in the rows having asterisk differ significantly from the normal values ($P<0.05$).

the groups 2 and 3 implying that vitamin E and selenium were having no effect on AchE activity.

Monoamine oxidase

The MAO activity (Table 2) increased significantly at 1 hr post-infusion and at 3 hr, it decreased to a level which was although higher but not significantly different from group 1. Thereafter, the MAO activity returned towards the normal baseline value in group 1. The MAO activity in group 3 too was significantly higher at 1 hr post-infusion as compared to the group 1 and thereafter, it was having no significant difference with the values in group 1 till 72 hr post-infusion. The level of this enzyme increased may be due to the increased norepinephrine levels during initial stages of shock, as this enzyme is responsible for the metabolism of catecholamines (Murray *et al*, 1996). There was no alteration in MAO activity with vitamin E and selenium supplementation.

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Cortisol and catecholamine profile in goats subjected to restraint and isolation stress

B G RUDRAMMA[1], V P VARSHNEY[2], SATISH KUMAR[3], JYOTHI BABU[4] and P C SANWAL[5]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

The effect of restraint and isolation stress (RIS) on plasma cortisol and catecholamines profile was studied in 12 adult Black Bengal female goats which were randomly alloted into experimental (n=6) and control (n=6) groups. Experimental goats were kept individually in metabolic trial cages and isolated for 6 hr on each of the six consecutive days, whereas the control goats remained undisturbed in the flock. Blood samples drawn at 0 (9.30 AM), 15,30,45, 60 min, 3rd and 6th hr of RIS on alternate days were analysed for plasma catecholamines using HPLC technique. Blood samples collected at 0, 3rd and 6th of RIS were analysed for plasma cortisol using ELISA technique. Later both the groups remained undisturbed in the flock for a period of 2 weeks (post-stress). Further blood samples were collected (at 9.30 AM) once in 4 days interval and analysed for plasma cortisol and catecholamines stress (RIS) significantly ($P<0.01$) increased plasma cortisol by 3rd hr and the level thereafter significantly ($P<0.01$) declined by 6th hr on 1st, 3rd and 5th day. Norepinephrine (NE) and epinephrine (E) concentration exhibited transcient and significant ($P<0.01$) increase by 15 min on 1st, 3rd and 5th day and subsequently declined significantly ($P<0.01$) up to 3rd hr, 60 min, 45 min on 1st, 3rd and 5th day, respectively, and remained similar up to 6th hr on respective days. The ratio of NE and E was similar and less than 1 up to 5 and 30 min on 1st, 3rd and 5th day respectively. Thereafter the ratio increased significantly ($P<0.01$) and remained similar up to 6th hr on 1st, 3rd and 5th day respectively. By 4th day of post-stress period, plasma cortisol and catecholamines were comparable with control goats. Thus the results of the present study suggested that active response to stress is due to activation of sympatho-adrenal-medullary axis (catecholamines) and passive response is due to hypothalamic-pituitary-adrenal axis (cortisol) activation and epinephrine is the principal catecholamine in response to stress in goats.

Key words: Catecholamines, Cortisol, Goat, Stress

There is no doubt that an environment with minimum stressors favours an efficient production in domestic farm animals and also ensures the well being of such species, but certain environmental or managemental conditions experienced by farm animals elicit a non-specific and stereotyped stress reaction. Activation of the stress system leads to behavioural and peripheral changes that improve the ability of the organism to adjust homeostasis and increase its changes for survival. There has been an exponential increase in knowledge regarding the interactions among the components of the stress system and between the stress system and other brain elements involved in the regulation of emotion, cognitive function and behaviour as well as with the axis responsible for reproduction, growth and immunity.

Data from the animal experimentations, tend to suggest that responses to different types of stressors are characterised by activation of hypothalamo-pituitary-adrenal axis as well as involvement of sympathetic nervous system. Stressors, depending upon the magnitude, have been linked to cause adverse effect which may be responsible for losses in animal productivity.

Experimentally restraint and isolation stress (Minton 1994), immobilization (Grimee and Wulfert 1985), confinement

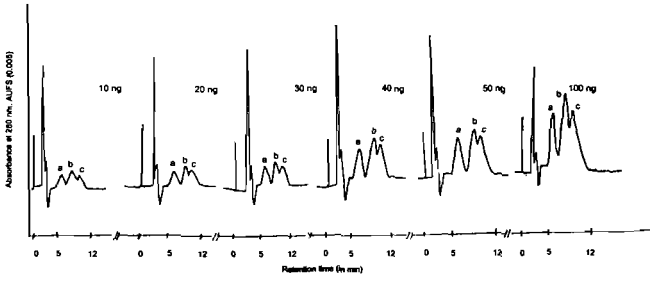

Fig. 1. Chromatogram of norepinephrine (a), epinephrine (b) and dihydroxybenzylamine (c) standards.

Present address: [1]C/o Dr N K S Gowda, NIANP, Adugodi, Bangalore 560 030.

[2]Principal Scientist, Animal Physiology Division, Principal Scientist, Biophysics.

[4]Technical Officer, IVRI, Palampur, Himachal Pradesh.

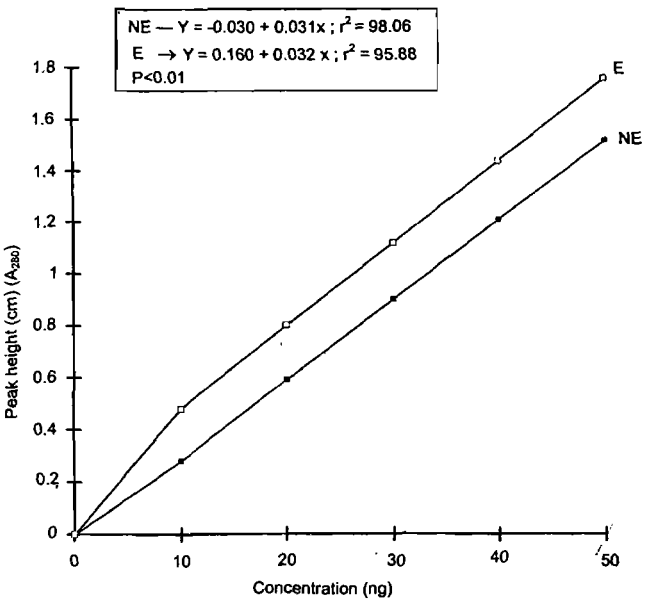

Fig.2. Standard calibration curve obtained for norepinephrine (NE) and epinephrine (E).

(Rasmussen and Malven 1983) and transport (New *et al.* 1996) are reported to induce stress in farm animals. However, very few comprehensive analysis of changes in plasma catecholamines and cortisol concentrations during experimental stress conditions in particular on goats is scarce although goats are an important resource and play an essential role in small holdings.

In the present investigation changes in plasma cortisol and catecholamines were measured in goats to ascertain their extent of involvement during 6 hr of restraint and isolation stress (RIS) for consecutive days.

MATERIALS AND METHODS

Animals and management

Twelve female adult (15-20 kg), healthy, Black Bengal goats were randomly divided into 2 groups of 6 animals each under experimental and control groups respectively. Animals were maintained under uniform management practices and were provided standard concentrate ration, adequate legumes and wheat *bhoosa*. Clean drinking water was offered twice daily.

The experiment was conducted during the month of November. Six goats of experimental groups were given restraint and isolation stress (RIS) for 6 hr daily for 6 consecutive days by placing them individually in metabolic trial cages, which were kept away from the visual and tactile contact of other goats. Control goats were housed in the animal shed along with other goats and remained undisturbed during the entire experimental period.

Collection and processing of blood samples

Blood samples were drawn at 15 min interval during the first hour of isolation for the estimation of catecholamines and then at 3rd and 6th hour for the estimation of the both cortisol and catecholamines on alternate days. During the post-stress period the experimental animals were mixed with control goats in the flock and blood samples were collected once in 4 days interval for 2 weeks. For the estimation of cortisol, blood samples were collected in heparinized saline, centrifuged and preserved at –20°C. For catecholamines, blood samples were collected in heparinized saline with sodium metabisulfite (0.5 mg/ml), immediately preserved in an ice container, centrifuged at 4° C and preserved at –196° C until assayed.

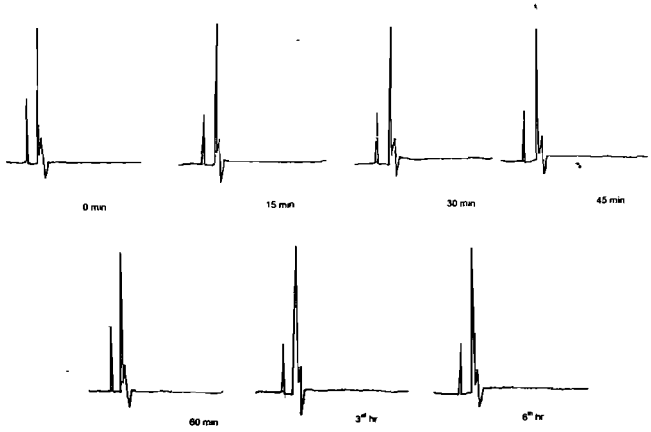

Fig.3. Plasma norepinephrine (NE) and epinephrine (E) concentration of female goats on day 1, 3 and 5.

Hormonal analysis

Cortisol: Plasma cortisol was estimated by ELISA technique. Plasma samples were added to microtitre plate supplied precoated with anticortisol antibodies. This was followed by addition of cortisol conjugated to horseradish peroxidase. Cortisol in the sample and the 1 conjugated to enzyme was allowed to compete for binding to antibody coated in microwell during specified incubation period. After specified time unbound conjugate was removed by washing the plate. Addition of chromogen (substrate) resulted in a colour product which was read at 450 nm after adding stop solution at specified time.

Catecholamines

Plasma catecholamines were estimated by high performance liquid chromatography, (HPLC) technique with UV detection (Leroy and Anthony 1977, Garcia *et al.* 1994). The HPLC system consisted of a Tracor double pump (model 955) with isocratic solvent system of sample/standard 50µl was injected into an analytical ODS (Lichrosorb, Rp 18, 4×250 mm size, 10µm) column via Rehodyne injector and monitored on a variable wavelength UV detector (Model LKB-2151) at 280 nm and absorbance range of 0.005 connected with a 2 channel recorder (ModeL LKB-2210). Flow rate (0.99 ml/min) befitting the need of the elution experiment was

Table 1a. Plasma cortisol concentration (ng/ml) during stress in Black Bengal female goats

Day	Control				Restrained and isolated			
	0 hr*	3 hr	6 hr	Ovarall average	0 hr+	3 hr	6 hr	Overall
1 st day	13.83±0.83	12.83±0.83	11.50±0.76	12.72±0.49	13.33^{c}±0.88	51.00^{aA}±1.52	29.33^{bA}±1.74	31.22^{A}±3.82
3 rd day	13.16±0.79	12.00±1.00	11.16±0.87	12.11±0.52	13.66^{c}±0.84	36.00^{aB}±1.65	24.83^{bB}±1.60	24.83^{B}±2 34
5th day	13.50±0.84	12.16±0.65	11.00±0.63	12.22±0.46	13.00^{c}±0.57	25.33^{aC}±0.88	20.33^{bB}±0.55	19.55^{C}±1.28
Overall hour/group	13.50±0.45	12.33±0.46	11.22±0.41	12.35±0.28	13.33^{c}±2.66	37.44^{a}±0.42	24.83^{b}±1.73	25.20±1.65
**average								

*Samples are collected at 9.30 AM; means ith different superscripts (abc), (**) in a row and column (ABC) different significantly (P<0.01).

Table 1b. Plasma cortisol concentration (ng/ml) during post-stress in Black Bengal female goats

Groups	Day			
	10th	14th	18th	22nd
Control	13.33±0.88	13.16±0.60	13.50±0.88	13.66±0.76
Restrained and isolated	13.16±0.79	13.66±0.55	13.66±0.49	13.50±0.76

employed. Chromatograms were recorded appropriately by synchronising the chart speed (2 mm/min) with the flow rate and 0.17 mon/litre of acetic acid buffer (pH -2.8; micro filtered and deaerated) was used as mobile phase. Norepinephrine and epinephrine bitartrate was used as standards and dihydroxy benzylamine was used as internal standard.

Sample preparation

Samples were prepared as per the method described by Ivan *et al.* (1981). To the aliquot of 1 ml frozen plasma sample, 10 mg of acid washed alumina was added and the volume brought to 1.5 ml with 0.5 M tris buffer (pH -8.6). Catecholamines were adsorbed to the alumina by shaking the tubes thoroughly for about 15 min. Once the alumina settled at the bottom of the tube, the supernatent was aspirated off. A single wash with 1.5 ml of deionized water was then given to the alumina, shaken and aspirated off thoroughly. Catecholamines were then deobserved from the alumina into 50µl of 0.2 M perchloric acid (60%). The tubes were then shaken thoroughly and centifuged at 15 000 rpm for 15 min. The clear perchloric acid supernatent was utilized for application to the HPLC system.

Sample assay

The alumina extracted acid supernatent (50µl) was injected into the column and chromatograms were recorded. The height of the peaks was estimated and catecholamines concentration was determined from the standard curve (Figs 1 and 2).

Statistical analysis

The data were subjected to the analysis of per Snedecor and Cochran (1967) and if the values were found significant, critical difference test was applied.

RESULTS AND DISCUSSION

Cortisol: The overall plasma concentration was significantly (P<0.01) higher in stress induced goats (25.20 ± 1.65 ng/ml) as compared to control animals (12.35 ± 0.28 ng/ml) (Table 1a). Interaction between days and groups was also significantly (P<0.01) different. Exposure to stress significantly (P<0.01) increased plasma cortisol concentration by 3rd hr which declined (P<0.01) by 6th hr. The concentration of plasma cortisol declined significantly (P<0.01) with the advancement of stress period from days 1 to 5. As early by 4th day post-stress period, plasma cortisol level was comparable in both the group of goats (Table 1b). Cortisol secretary response to RIS (social and psychological) has been much studied in sheep (Apple *et al.* 1993, Minton 1994) when compared to other farm animals in general and goats in particular. In this study plasma cortisol level increased significantly (P<0.01) by 3rd hr and declined significantly (P<0.01) by the 6th hr on 1st, 3rd and 5th day following exposure to RIS. With the advancement of stress period from day 1 to day 5 the concentration of plasma cortisol declined

Table 2. Plasma norepinephrine concentrations (ng/ml) during stress in Black Bengal female goats

	1st day	3rd day	5th day
0 min	ND	ND	ND
15 min	12.72 ± 0.28^{A}	10.08 ± 0.08^{A}	10.00 ± 0.01^{A}
30 min	9.40 ± 0.60^{B}	6.76 ± 0.11^{B}	4.32 ± 0.33^{B}
45 min	5.86 ± 0.65^{C}	3.38 ± 0.35^{C}	1.30 ± 0.08^{C}
60 min	3.04 ± 0.03^{D}	1.16 ± 0.02^{D}	1.26 ± 0.06^{C}
3 hr	1.18 ± 0.01^{E}	1.16 ± 0.02^{D}	1.26 ± 0.06^{C}
6 hr	1.18 ± 0.01^{E}	1.16 ± 0.02^{D}	1.26 ± 0.06^{C}
Overall day average	5.56 ± 0.81^{a}	3.94 ± 0.63^{b}	3.23 ± 0.60^{C}

ND-Not detectable means with different superscripts (ABCDE) in a column and row (abc) differed significantly (P<0.01). Plasma norepinephrine concentrations during post-stress (day 10, 14, 18, 22) in Black Bengal female goats was not detectable.

significantly (P<0.01). By 4th day of post stress period, cortisol level was comparable with control goats. Similarly Sanhouri *et al.* (1989) and Nwe *et al.* (1996) observed rapid elevation of cortisol level by 15 min and 30 min after the start which remained elevated throughout the transportation stress (motorized van transportation or Noisy trolley transportation and truck journey) in goats. In pigs also a rapid increase in plasma cortisol level after transportation is consistent with these findings. The initial rise in the cortisol level is due to activation of hypothalamo-pituitary-adrenal axis (HPA) and decrease in the cortisol secretary response is as a result of habituation of the animals to repeated exposure to the same stress. This observation is similar to the finding reported by Kent and Ewbank (1983) and Kent and Ewbank (1986). Crotisol response to RIS in sheep have revealed similar findings (Coppinger *et al.* 1991, Minton *et al.* 1992, Apple *et al.* 1993). However, on contrary to these findings data have been obtained in sheep and pig, indicating diminution of hypothalamo-pituitary-adrenal axis response is not a consistent feature in different species of livestock and differing types, frequencies and duration of stressors (Niezgoda *et al.* 1987, Klemcke 1994, Rushen *et al.* 1993).

Catecholamines: Plasma norepinephrine (NE) concentration exhibited transcient increase (P<0.01) by 15 min of exposure to stress and subsequently declined significantly (P<0.01) upto 3rd hour, 60 min and 45 min on 1st, 3rd and 5th day, respectively, and remained similar up to 6th hr on, respective, days (Table 2). Irrespective of time intervals the over all day concentration of plasma NE declined (P<0.1) from day 1 (5.56 ± 0.81 ng/ml) to day 5 (3.23 ± 0.6 ng/ml) plasma epinephrine (E) concentration also showed a similar transient increase (P<0.01) by 15 min of exposure to stress and thereafter decreased (P<0.01) up to 3rd hr, 60 min and 45 min on 1st, 3rd and 5th day, respectively, and remained similar up to 6th hr on respective days. The overall day concentration of plasma epinephrine declined significantly (P<0.01) from day 1 (6.05 ± 0.92 ng/ml) to day 5 (3.59 ± 0.71 ng/ml). Plasma catecholamines concentration is significantly elevated in restrained and isolated goats and maintained at a higher concentration over the entire 6 hr stress period. In control goats the concentration of both E and NE was observed to be below the detectable limit of the assay (Fig. 3). The levels of E and NE were also not detectable as early as 4th day of post stress indicating that impact of stress does not exist after it termination. The peak level of E and NE occurred at 15 min. after the start of RIS and the concentration of E at peak was relatively and absolutely higher as compared with that of NE. This observation is in agreement with the results obtained by Nwe *et al.* (1996) who showed that plasma E is the principal catecholamine observed immediately after the start of the transportation stress. The magnitude of increase in E and NE concentration in the present study was higher than those recorded during transportation stress by Nwe *et al.* (1996). However, these workers did not record any significant change in the levels of norepinephrine throughout the transportation stress. The time course of plasma E response with its immediate and sustained increase in the present study was similar to that observed by Nwe *et al.* (1996) and Dalin *et al.* (1993) in pigs subjected to transportation stress. However, contrary to these findings Antonie *et al.* (1995) observed that the concentration of plasma NE was relatively and absolutely higher in pigs given snare restraint for 5 min suggesting that NE is the principal catecholamine in acute stress response.

In the control goats, plasma NE and E concentrations were found below the detectable limit (<1.00 ng/ml) of the assay at different time intervals in a day and on different days of the experiment. (Fig. 3). As early by 4th day of post-stress period, plasma catecholamines levels were found below the detectable level as comparable with control goats (Tables 2 and 3).

Table 3. Plasma epinephrine concentrations (ng/ml) during stress in Black Bengal female goats

	1st day	3rd day	5th day
0 min	ND	ND	ND
15 min	14.00 ± 0.01^{A}	11.72 ± 0.40^{A}	11.28 ± 0.27^{A}
30 min	10.52 ± 0.53^{C}	8.05 ± 0.27^{B}	5.88 ± 0.23^{B}
45 min	7.16 ± 0.21^{C}	4.28 ± 0.40^{C}	1.18 ± 0.11^{C}
60 min	2.60 ± 0.06^{D}	1.02 ± 0.02^{D}	1.08 ± 0.03^{C}
3 hr	1.02 ± 0.02^{E}	1.02 ± 0.02^{D}	1.08 ± 0.03^{C}
6 hr	1.02 ± 0.02^{E}	1.02 ± 0.02^{D}	1.08 ± 0.03^{C}
Overall day average	6.05 ± 0.92^{a}	4.52 ± 0.76^{b}	3.59 ± 0.71^{c}

ND- Not detectable. Means with different superscripts (ABCDE) in a column and row (abc) differed significantly (P<0.01). Plasma epinephrine concentrations during post-stress period (day 10, 14, 18, 22) in Black Bengal female goats were found, undetectable.

Norepinephrine and epinephrine ratio

The ratio of NE and E was found similar and less than 1

Table 4. Plasma norepinephrine: epinephrine ratio during stress in Black Bengal female goats

Time	1st day	3rd day	5th day
0 min	ND	ND	ND
15 min	0.89 ± 0.01^{B}	0.85 ± 0.03^{B}	0.88 ± 0.02^{B}
30 min	0.88 ± 0.01^{B}	0.84 ± 0.03^{B}	0.72 ± 0.04^{B}
45 min	0.83 ± 0.08^{B}	0.78 ± 0.02^{B}	1.11 ± 0.03^{A}
60 min	1.17 ± 0.02^{A}	1.13 ± 0.02^{A}	1.16 ± 0.03^{A}
3 hr	1.15 ± 0.02^{A}	1.13 ± 0.02^{A}	1.16 ± 0.03^{A}
6 hr	1.15 ± 0.02^{A}	1.13 ± 0.02^{A}	1.16 ± 0.03^{A}
Overall day average	1.01 ± 0.03^{ab}	0.98 ± 0.03^{b}	1.06 ± 0.04^{a}

ND- Not detectable. means with different superscripts (ab) in a row differ significantly (P<0.05). Mean with different superscripts (AB) in a column differ significantly (P<0.01).

upto 45 min, 45 min and 30 min, on 1st, 3rd and 5th day respectively (Table 4). Thereafter the ratio increased (>1) significantly (P<0.01) and remained similar up to 6th hr on all the days. The ratio of NE and E was similar and less than 1 up to 45 min on first and 3rd day and thereafter the ratio increased (>1) significantly (P<0.01) and remained consistent up to 6th hr of RIS (Table 4). It indicates that initially epinephrine is secreted more after 15 min of the start of the experiment followed by the increased NE production.

It is concluded that experimental induction of RIS to goats is associated with the activation of SAM axis (active response) and also the HPA axis (passive response). Consequent and gradual decrease in plasma cortisol and catecholamines (E and NE) concentration with advancement of stress period, suggest adaptive response of animals and epinephrine is the principal catecholamine during stress in goats.

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Production and characterization of monoclonal antibody against outer membrane protein of *Pasteurella multocida**

RASHMI SINGH[1], ARVIND KUMAR[2] and M C GOEL[3]

CCS Haryana Agricultural University, Hisar, Haryana 125 004 India

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The importance of monoclonal antibodies (mAbs) in the development of vaccines against various infectious diseases is well established. These are used to identify, isolate or purify antigens and also to define the role of specific antigens in diagnosis, classification and immunity to infection (Lu *et al.* 1991). Haemorrhagic septicemia (HS) is a per acute disease of bovine population caused by *Pasteurella multocida* Carter's type B. The disease is enzootic in India and it causes heavy mortality. Use of conventional vaccines [Oil adjuvant vaccine (OAV) and alum precipitated vaccine (APV)] controlled it to a great extent but these vaccines have got their own limitations. An improved vaccine is thus needed which is more potent, easier to inject and have a longer period of immunity. Subunit vaccines can be the break needed for control of spread of HS in the population. For this, more potent immunogens are needed which are provided in the form of outer membrane proteins (OMPs) on the bacterial outer membrane (Osborn and Wu 1980). The protein profiles of *P. multocida* (Carter B) isolates from animals with HS are relatively homogenous with major bands having molecular mass between 27 kD to 47 kD (Ruffolo and Adler 1996). Selection of *P. multocida* strains with high OMP production under modified cultural conditions could enhance vaccine potency (Srivastava 1998). A major OMP of molecular weight 32 kD was identified in *P. multocida* Carter B found prevalent in Asia and North America (Johnson *et al.* 1991). Therefore its presence in Carter B and absence in other isolates (Carter E) makes it a marker antigen and hence a potential candidate for identification of *P. multocida* type B strains. It also plays an immunogenic role against the infection (Dawkins *et al.* 1991). It can hence be used for preparation of subunit vaccine for HS and therefore production of mAbs against this protein would prove useful in identifying, isolating, purifying and to define its role in diagnosis and immunity to infection. Keeping in view, the present study was under taken to produce and characterize mAb against 32 kD OMP of *P. multocida* P52 strain.

The mAb against OMP of *P. multocida* was raised as per the basic protocol of Kohler and Milstein (1975) with modification as described by Campbell (1984). For this purified preparation of 32 kD OMP was used as the antigen.

Immunization of BALB/c mice

Two BALB/c mice of 8-10 weeks of age were immunized as per immunization schedule of Lu *et al.* (1991) with slight modifications. Animals were primed by a s/c injection of 75µg of OMP antigen emulsified in complete Freund's adjuvant. A second s/c injection of 75µg of OMP antigen emulsified in incomplete Freund's adjuvant was administered 2 weeks later. Third injection of 75µg of OMP antigen without adjuvant was administered i/p 7days later.

Myeloma cells

SP2/0 myeloma cells were seeded at a concentration of 1-2× 10^5 cells/ml in RPMI-1640 medium, which was added to the level of 0.4ml/cm^2 of the flask area. Cells were used in the log phase of growth cycle for the fusion.

Spleen cells

The spleen cells were harvested from the mouse with high antibody titer 72 hr after last injection in RPMI-1640 medium. The spleen cell count was taken approximately as 10^8 cells.

Fusion procedure

The myeloma cells and the spleen cells were mixed together in 1: 10 ratio. Trypan blue dye exclusion test was used to assay the viability of the cells. Fusion was done using 1ml of polyethyleneglycol-1500 (50%) solution. The cell pellet was gently suspended in 50ml of the hybridoma medium (RPMI-1640, 20% foetal calf serum, 1% L-glutamine and 2% hypoxanthine, aminopterin, thymidine (HAT) -50X). The cell suspension was pipetted @ 100µl/well into five 96 – well

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Present address: [1]Junior Research Officer (Vaccinology), Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, GBPUAT, Pantnagar 263 145. [2]Professor, Department of Veterinary Microbiology, [3]Formerly Dean PGS, College of Veterinary Sciences.

tissue culture plates. The spleen cells from a Swiss albino mouse were used as feeder cells for the hybridomas. The plates were incubated in humidified 5% CO_2 and 95% air incubator at 37°C.

Maintenance of hybrids

After 48 hr of fusion, 100µl of hybridoma medium was added to each well of the plate. Then after every 48 hr, 100µl of supernatant was removed and fresh hybridoma medium was added.

Screening of wells

The supernatant from the wells containing hybridoma was screened for the presence of the specific antibody by indirect solid phase enzyme linked immunosorbent assay (ELISA). The test was first standardized using the hyper immune serum raised against the whole organism in mice. Supernatant (100µl) was added in each antigen coated well (17 µg/ ml OMP) and incubated overnight at 4°C. Horse radish peroxidase conjugate anti-mouse serum was used as second antibody and orthophenylene-diamine was used as substrate. The positive hybridomas were screened 4 more times for the antibody production.

Amplification of hybridomas

The ELISA positive hybridomas were amplified. For this 100µl of cell suspension from each selected well of 96-well tissue culture plate was transferred to 12- well tissue culture plate in 1 ml media. After obtaining confluent growth, cells were transferred to a 6- well culture plate in 3 ml culture volume. From 6- well plate, cells were transferred to a 25 cm^2 tissue culture flask in 10 ml volume and finally to 80 cm^2 tissue culture flask in 30 ml culture volume.

Cryopreservation of hybridomas

The hybridoma culture having above 95% viable cells were pelleted by centrifugation and cell pellet was resuspended in a cell cryopreservation medium containing 10% dimethyl sulphoxide. The cell concentration was kept as 5×10^6 cells/ ml. The aliquots of cells were first kept at –70°C overnight and then shifted into liquid nitrogen.

Production of mAbs

In cell culture supernatant: The cells were seeded at 1×10^5 cells/ml in a 25 cm^2 flask. When cells obtained optimum concentration, they were transferred to a 80 cm^2 tissue culture flask in 30 ml culture volume and were over-grown till the culture showed 50% degeneration of the cells. Cells were brought to the suspension by agitation, centrifuged and the supernatant was collected, aliquoted and stored at –20°C. It was titrated against OMP antigen in indirect solid phase ELISA.

In ascitic fluid in mice: The hybridoma was grown as peritoneal tumor in 2 BALB/c mice and ascitic fluid was collected as a source of antibody. After about 7-10 days of priming with pristine each mouse was injected 5×10^6 cells i/p and ascitic fluid was tapped by introducing sterile needle into peritoneal cavity. The fluid was clarified by low speed centrifugation, stored frozen at –20°C and titrated by ELISA.

Characterization of mAb

The mAb was characterized for its specificity, isotype and its ability to protect mice against challenge with *P. multocida.*

Immunoblotting: The OMP antigen resolved by SDS-PAGE was electrophoretically transferred from the gel to a 0.45 mm pore size nitrocellulose membrane (NCM) in semi-dry electrophoretic apparatus.

Isotyping: The isotype of mAb was determined using indirect solid phase ELISA using goat anti-mouse isotype specific antisera.

Passive mouse protection test (PMPT): Lethal dose fifty (LD_{50}) was calculated as per Reed and Muench (1938). For PMPT, anti-OMP as ascitic was given i/p in a batch of 3-4 mice and the mice were challenged on third day. The time to death of those succumbed to challenge was also recorded.

Immunization and fusion

The antibody titer of the mouse immunized with *P multocida* was 3.9 log^{10}. When spleen cells (10^8) from this mouse were allowed to fuse with 10^7 myeloma cells (10: 1) and plated in five 96-tissue cultured plates, 356 wells showed the presence of hybrids. Fusion frequency was calculated as 356 hybrids/10^8 lymphocytes or 74.16% of total wells.

Screen assay

Hybrid cell supernatant giving an OD value 1.5-times more than that of control well was taken as positive for mAb production. Out of 120-hybrid cell supernatants screened, 67 for positive for the presence of mAbs. Of these 67 hybrids, 14 hybrids having an OD value above 0.3 were selected and finally 1 hybride 5G3 was amplified.

In vitro *and* in vivo *production of mAb*

The titer of mAb in cell culture supernatant and ascitic fluid of 5G3 hybrid was a 2.9 log^{10} and 4.2 log^{10} respectively.

Characterization of mAb

Immunoblotting: OMP antigen (4.25 µg) was resolved in 11% polyacrylamide gel and transferred to NCM, it was incubated with 1: 100 dilution of polyclonal serum, 5G3 hybridoma supernatant, ascitic fluid of 5G3 hybridoma in dilution of 1: 100 and 1: 10 respectively. On developing the blot with diaminobenzidene 32 kD OMP reacted only with polyclonal serum. However, no reaction was observed with mAb present in cell culture supernatant and ascitic fluid. Failure of mAb to react with any protein band could have indicated that epitope recognized by mAb was presumably of conformational structure and did not renature after transfer to NCM.

Isotyping: The mAb belonging to IgG_1 serum gave a mean

OD value of 0.58 at 492nm.

PMPT: The LD_{50} of *P. multocida* was 0.1 ml of $10^{-6.6}$ dilution (5 colony forming units). All the mice were immunized with mAb or anti-pasteurella hyper immune serum and challenged 1hr after immunization. 25% of group 2 (0.2 ml ascitic fluid) mice survived the challenge and increase in mean time to death from 1 and 3.5 days was observed. Hyper immune serum against whole organism in mice and rabbit afforded 100% protection. Normal mice serum was used as negative control.

It has been previously demonstrated that principal response of mice to *P. multocida* type B; OAV and bacterin was in the IgG class. Moreover, there was strong and highly significant relationship between anti-pasteurella IgG antibody levels and passive protection afforded by passive transferred serum in mice (Dawkin *et al.* 1991). Ability of mAb to partially protect could, therefore, be ascribed to its isotype and specificity to 32 kD OMP. The 32kD OMP is not just a marker protein for the Asian HS isolates but possibly a definitive role in the protection against *P. multocida* infection. Additional studies will be required to establish its biochemical characteristic, surface location and extent of protection provided by active immunization.

It is hoped that availability of this mAb will facilitate the isolation and purification of the 32kD OMP from *P. multocida* causing HS and development of an effective subunit vaccine against this infection.

SUMMARY

Spleen cells of mouse immunized with 32 kD OMP antigen were allowed to fuse with SP2/0 myeloma cells to obtain 356 hybrids. Out of 120-hybrid cell supernatant, 67 were positive for antibody production. The cell culture supernatant and ascitic fluid was positive for mAb by ELISA and they belonged to IgG3 subclass. Also it provided partial protection when mice were passively immunized and an increase in mean time of death was observed from 1 to 3.5 days.

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Antibiogram and bacterial flora of pyogenic infections of domestic animals in Kashmir valley

S A WANI[1], M A BHAT[2] and A S BACH[3]

SK University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shuhama (Alusteng), Srinagar, Jammu and Kashmir 190 006 India

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A great number of animals presented to veterinarians have localized pyogenic infections, many of them are due to trauma. Even if most of them have surgical outcome, the initial treatment is often medical, especially if the exact location of the lesion cannot be identified. When the antibacterial agents are used systemically to treat the local suppurative lesion, the choice of an antimicrobial agent should take into account the probable identity and the likely sensitivity of the causative organism. Present investigation was therefore undertaken to study the causative organisms of pyogenic infections of domestic animals as well as to know their sensitivity pattern to different antibacterials.

A total of 45 pus samples were collected from various domestic animals (Table 1) presented to the Veterinary Polyclinic of this college for treatment. Pus or exudate from infected wound was usually sampled by means of sterile swabs, which were well soaked in the exudate. Sometimes, the pus was obtained using a syringe and transferred to a sterile tube or screw capped vial. The specimens were immediately transferred to the laboratory.

Table 1. Distribution of various bacterial isolates from pyogenic infections of different animal species

Seecies	Bovine (31)	Equine (06)	Caprine (04)	Ovine (02)	Canine (02)
Staphylococcus spp.	4	1	1	1	1
Streptococcus spp.	6	1	0	1	0
Pasteurella spp.	1	0	1	0	0
Escherichia spp.	3	2	0	1	0
Klebsiella spp.	1	1	0	0	1
Pseudomonas spp.	1	0	0	0	0
Morganella spp.	1	1	0	0	0
Edwardsiella spp.	0	1	0	0	0
Corynebacterium spp.	0	0	1	0	0
Total	17	7	3	3	2

Figures in parentheses indicate total number of pus samples collected from each species of animal.

Present address: [1]Senior Scientist-cum-Head, [2]Assistant Professor-cum-Junior Scientist, Division of Microbiology and Immunology, [3]Professor and Head.

The samples were directly cultured on blood agar (BA) MacConkey's lactose agar (MLA) plates and incubated at 37°C up to 3 days for isolation of causative bacteria, if any. The isolated colonies were picked up in nutrient agar or blood agar slants as pure cultures and subjected to standard morphological, biochemical tests for identification of organism (Buchanan and Gibbon 1994, Carter *et al.* 1995, Collee *et al.* 1996). The *Staphylococcus* cultures were subjected to coagulase test and all coagulase positive cultures were sent for phage typing to Staphylococcal Phage Typing Centre, Maulana Azad Medical College, New Delhi 110 002. All the confirmed *E. coli* isolates were got serotyped from National Salmonella and Escherichia Centre, Research Institute, Kasauli, Himachal Pradesh 173 204. The *in vitro* susceptibility of isolates of antimicrobial agents was determined by single disc diffusion method described by Bauer *et al.* (1966). The antimicrobial discs used for the study were gentamicin, tetracycline, erythromycin, ciprofloxacin, chloramphenicol, ampicillin/cloxacillin, co-trimoxazole and norfloxacin, marketed by HiMedia, Mumbai (India). Interpretation of isolates as sensitive and resistant was made as per the interpretation chart of the supplier.

Out of 45 pus samples 23 were positive for different bacterial organisms. The organisms isolated were *Staphylococcus aureus, Streptococcus* spp., *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Pasteurella multocida, Pasteurella haemolytica, Pseudomonas aeruginosa, Morganella morganii, Edwardsiella ictaluri* and *Corynebacterium* spp. Isolation of these organisms from pus samples is in agreement with the existing literature (Carter *et al.* 1995).

Fifteen samples yielded single isolates while 7 samples yielded mixed infection of 2 organisms and 1 sample revealed the presence of 3 organisms. From the Table 1, it is evident

that *Staphylococcus* spp. and *Streptococcus* spp. (25% each) were most prevalent followed by *Escherichia coli* (18.75%), *Klebsiella* spp. (9.37%), *Morganella* spp. and *Pasteurella* spp. (6.25% each). While as *Pseudomonas* spp, *Edwardsiella* spp., *Corynebacterium* spp. (3.12% each) were least prevalent. *Pasteurella multocida* was isolated from a goat with multiple neck abscesses. A total of 8 staphylococcal isolates were recovered of which 6 were coagulase positive. *Staphylococcus aureus* strains were typed by the group of standard phages of RTD × 100. Out of 6 *Staphylococcus aureus* isolates 4 were untypable while as one was lysed with phage 29/80 and another by phage 54, belonging to human lytic phage groups 1 and 3 respectively. Out of 6 *E. coli* isolates 1 was untypable and the remaining 5 belonged to O8, O9, 025, 111 and O113 serotypes. Serogroup O8 and O25 are enterotoxigenic *Escherichia coli* (ETEC; Collee *et al.* 1996). O111 serotype is enteropathogenic. *Escherichia coli* (EPEC) and has never been recorded in normal faeces (Orskov and Orskov 1992).

The results of *in vitro* antibiotic sensitivity tests are presented in the Table 2. Majority of isolates was sensitive to gentamicin (77.4%), followed by chloramphenicol (64.5%), ciprofloxacin (61.2%), norfloxacin (51.6%) and tetracycline (41.9%). While as majority of isolates were resistant to ampicillin/cloxacillin, co-trimoxazole and erythomycin. *Pseudomonas aeruginosa* and *E. coli* O25 serotype isolated from bovine wound infection were resistant to all the antibiotics used in the test, while as *E. coli* O8 serotype isolated from bovine neck abscess was resistant to all antibiotic applied except chloramphenicol. Similarly, 1 *Corynebacterium* isolate was resistant to all antibacterials except tetracycline. The highest degree of drug resistance emerged against ampicillin/cloxacillin and co-trimoxazole. This might be due to the fact that perhaps these preparations are most extensively and indiscriminately used in veterinary practice. Emergence of many multidrug resistant strains of bacteria during the study is alarming and strongly suggests the judicious use of antibacterials in clinical applications.

SUMMARY

Present investigation reports antibiogram and bacterial flora of domestic animals in Kashmir valley. Out of 45 pyogenic samples 23 samples revealed 32 bacterial isolates (*Staphylococcus aureus, Streptococcus, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Pasteurella multocida, Pasteurella haemolytica, Pseudomonas aeruginosa, Morganella morganii, Edwardsiella ictaluri* and *Corynebacterium*). *Staphylococcus* spp. and *Streptococcus* spp. were most prevalent organisms with an incidence of 25% while *Pseudomonas* spp., *Edwardsiella* spp., *Corynebacterium* spp. were least prevalent with a prevalence of

Table 2. *In-vitro* antibiotic sensitivity patterns of bacterial isolates from pus samples

Bacterial spp.	Total no. of isolates	No. of isolates sensitie to							
		Ax	E	C	Cf	G	Co	Nx	T
Staphylococcus spp.	8	4	1	8	5	8	1	5	3
		(50)	(12.5)	(100)	(62.5)	(100)	(12.5)	(62.5)	(37.5)
Streptococcus spp.	8	3	1	6	8	8	3	6	7
		(37.5)	(12.5)	(75)	(100)	(100)	(37.5)	(75)	(87.5)
Escherichia spp.	6	0	0	5	2	4	2	1	1
		(0)	(0)	(83.3)	(33.3)	(66.6)	(33.3)	(16.6)	(16.6)
Klebsiella spp.	3	0	1	0	2	1	0	2	0
		(0)	(33.3)	(0)	(66.6)	(33.3)	(0)	(66.6)	(0)
Pasteurella spp.	2	0	0	0	1	1	0	1	1
		(0)	(0)	(0)	(50)	(50)	(0)	(50)	(50)
Pseudomonas spp.	1	0	0	0	0	0	0	0	0
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Morganella spp.	2	0	0	0	1	1	1	0	0
		(0)	(0)	(0)	(50)	(50)	(50)	(0)	(0)
Edwardsiella spp.	1	0	1	1	0	1	0	1	0
		(0)	(0)	(0)	(0)	(0)	(0)	(100)	(0)
Corynebacterium spp.	1	0	0	0	0	0	0	0	1
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(100)
Total	32	7	4	20	19	24	7	16	13
		(21.8)	(12.5)	(62.5)	(59.3)	(75.0)	(21.8)	(50.0)	(40.6)

Figures in parentheses indicate percentage. Ax, Ampicillin/coloxacillin; E, erythomycin; C, chloramphenicol; Cf, ciprofloxacin; G, gentamicin; Co, co-trimoxazole; Nx, norfloxacin; T, tetracycline.

3.12% each. Majority of isolates (77.4%) was sensitive to gentamicin while least number of isolates (41.9%) were sensitive to tetracycline.

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Detection and differentiation of mycoplasma from bacteria and fungi by polymerase chain reaction

SANJOY DAS[1], VIJENDRA P SINGH[2], M MANOJ KUMAR[3] and V P SINGH[4]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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Mycoplasma, the smallest self-replicating, prokaryote causes various disease conditions, viz. contagious bovine pleuropneumonia (CBPP), contagious caprine pleuropneumonia (CCPP), mastitis, arthritis, infectious keratoconjunctivitis, contagious agalactia and genital disorders in different domestic animals causing heavy economic losses (Nicolet 1994, Gunning and Shephered 1996). It is also present as a common contaminant in cell cultures used for viral propagation (Barile 1979).

The routine detection of mycoplasmas is generally carried out by isolation of the organisms and serological tests. The isolation of the organisms is very tedious, laborious and time consuming (Sachse *et al.* 1993), while the serological tests are not very sensitive and specific. Moreover, a higher antibody titre is also required for serological detection, which is generally not present in subclinical and chronic infections (Simecka *et al.* 1992). Recent development of molecular techniques, viz. polymerase chain reaction (PCR), restriction endonuclease analysis, DNA probes, have overcome this problem to a great extent. Among these techniques, PCR is the most sensitive and can detect the subclinical and chronic infections, when organisms are present in a very low number (Kuppeveld *et al.* 1992, Johansson *et al.* 1996 and Ghadersohi *et al.* 1999). The present study was intended to detect the different strains of mycoplasma and also differentiate these organisms from bacteria and fungi by specific PCR assay.

The 14 strains of mycoplasma, 18 strains of bacteria and 3 strains of fungi used in this study are listed in Table 1. These mycoplasma strains were procured from the National Referral Laboratory on Mycoplasma while bacterial and fungal strains from Division of Bacteriology and Mycology, IVRI, Izatnagar. The strains of *Mycoplasma mycoides* cluster viz. *M. mycoides* subsp. *capri*, *M. mycoides* subsp. *mycoides* SC, *M. mycoides* subsp. *mycoides* LC, *M. capricolum* subsp. *capricolum*, *M. capricolum* subsp. *capripneumoniae*, *M.* sp.

Present address: [1]Ph.D Scholar, [2]Senior Scientist and i/c National Referral Lab on Mycoplasma, [3]Research Associate, [4]Formerly Principal Scientist. National Referral Laboratory on Mycoplasma, Division of Bacteriology and Mycology.

Table 1. Mycoplasma, bacterial and fungal strains

Mycoplasma strains	
1.	*M. mycoides* subsp. *capri* (PG3) NCTC 10137
2.	*M. mycoides* subsp. *capri* (Bhuj)
3.	*M. mycoides* subsp. *mycoides* LC (NCVP -2/99)
4.	*M. mycoides* subsp. *mycoides* LC (Y-Goat) NCTC 11706
5.	*M. mycoides* subsp. *mycoides* SC (PG1) NCTC 10114
6.	*M. mycoides* subsp. *mycoides* SC (NCVP - 1/86)
7.	*M. capricolum* subsp. *capricolum* (California kid) NCTC 10154
8.	*M. capricolum* subsp. *carpricolum* (BMET)
9.	*M. capricolum* subsp. *capripneumoniae* (F.38) NCTC 10192
10.	*M.* sp. bovine group 7 (PG 50) NCTC 10133
11.	*M. bovis* (NC 317)
12.	*M. agalactiae* (RPNS 216)
13.	*M. agalactiae* (RPNS 200)
14.	*Acholeplasma laidlawii* (NC 313)
Bacterial and fungal strains	
1.	*Pasteurella multocida* (B: 2) P52
2.	*Pasteurella multocida* (A: 1)
3.	*Pasteurella multocida* (D: 1)
4.	*Pasteurella multocida* (F: 3)
5.	*Staphylococcus* spp.
6.	*Streptococcus pyogenes*
7.	*Corynebacterium pyogenes*
8.	*Bacillus cereus*
9.	*Pseudomonas* spp.
10.	*Escherichia coli*
11.	*Enterobacter agglomerans*
12.	*Listeria monocytogenes*
13.	*Klebsiella pneumoniae*
14.	*Salmonella* Typhimurium
15.	*Salmonella* Abortus equi
16.	*Salmonella* Lille
17.	*Salmonella* Cholerae suis
18.	*Salmonella* Neighton
19.	*Aspergillus flavus*
20.	*Aspergillus fumigatus*
21.	*Aspergillus parasiticus*

bovine group 7 and *Acholeplasma laidlawii* were grown in mycoplasma liquid media as described by Carmichael *et al.* (1972) whereas, B medium (Ernö and Stipkovits 1973) was used for the growth of *M. bovis* and *M.agalactiae.* However, all the bacterial strains used in present study were grown in BHI broth while the species of *Aspergillus* in Sabouraud's dextrose agar medium.

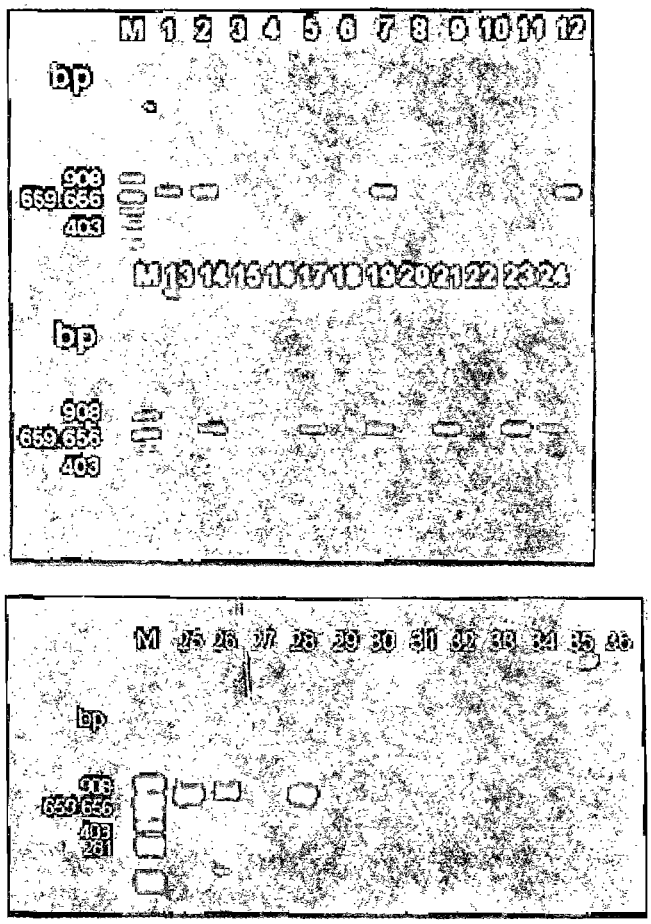

Fig. 1. Mycoplasma group specific PCR using primer GPO-1/ MGSO: Lane M: Marker (pBR 322 DNA digested with *Alu* I), Lane 1: *M. mycoides* subsp. *mycoides* SC (PG1), Lane 2: *M. mycoides* subsp. *mycoides* SC (NCVP - 1/86), Lane 3: *Pasteurella multocida* (B: 2), Lane 4: *P. multocida* (A: 1), Lane 5: *P. multocida* (D: 1), Lane 6: *P. multocida* (F: 3), Lane 7: *M. bovis* (NC 317), Lane 8: *Staphylococcus* sp., Lane 9: *Streptococcus pyogenes,* Lane 10: *Corynebacterium pyogenes,* Lane 11: *Bacillus cereus,* Lane 12: *M.* sp. bovine group 7 (PG 50), Lane 13: *Pseudomonas* sp., Lane 14: *M capricolum* subsp. *capripneumoniae* (F-38), Lane 15: *E. coli,* Lane 16: *M. mycoides* subsp. *mycoides* LC (Y-Goat), Lane 17: *M. mycoides* subsp. *mycoides* LC (NCVP - 2/99), Lane 18: *Enterobacter agglomerans,* Lane 19: *M. mycoides* subsp. *capri* (PG 3), Lane 20: *Listeria monocytogenes,* Lane 21: *M. mycoides* subsp. *capri* (Bhuj), Lane 22: *Klebsiella pneumoniae,* Lane 23: *M. capricolum* subsp. *capricolum* (Calif. kid), Lane 24: *M. capricolum* subsp. *capricolum* (BMET), Lane 25: *M. agalactiae* (RPNS 216), Lane 26: *M. agalactiae* (RPNS 200), Lane 27: *Salmonella* Typhimurium, Lane 28: *Acholeplasma laidlawii* (NC 313), Lane 29: *Salmonella* Abortus equii, Lane 30: *Salmonella* Lille, Lane 31: *Salmonella* Neighton, Lane 33: *A. flavus,* Lane 34: *A. fumigatus,* Lane 35: *A. parasiticus,* Lane 36: Negative control (No DNA).

Isolation of genomic DNA

The genomic DNA of all the strains were isolated as per the protocol of Wilson (1987) and the concentration of genomic DNA was finally adjusted to 10 ng/µl of DNA.

PCR assay

The PCR assay was performed using the genomic DNA of mycoplasma, acholeplasma, bacteria and lysate of fungi as template along with mycoplasma group specific primers GPO-1 [5'-ACT CCT ACG GGA GGC AGC AGTA - 3'] and MGSO [5' - TGC ACC ATC TGT CAC TCT GTT AAC CTC - 3'] (Kuppeveld *et al.* 1992). The PCR reaction was performed in a thermocycler using a programme consisting of an initial denaturation of 94° C for 2 min, followed by 30 cycles of denaturation (94° C for 45 sec), annealing (55° C for 1 min) and extension (72° C for 1 min). Final extension was carried out at 72° C for 5 min.

The PCR products were detected on 1.4% agarose gel electrophoresis along with the DNA marker (pBR 322 digested with *Alu* I enzyme) and then visualised and photographed under UV-gel documentation system.The sensitivity of the PCR assay was estimated using different 10-fold dilutions of template DNA from 100 ng to 100 fg.

In the present study, all the 14 strains of mycoplasma and acholeplasma yielded a 715 bp long fragment of 16S rRNA gene with GPO-1 and MGSO primers while none of the strains of bacteria and fungi yielded any amplified product (Fig. 1).

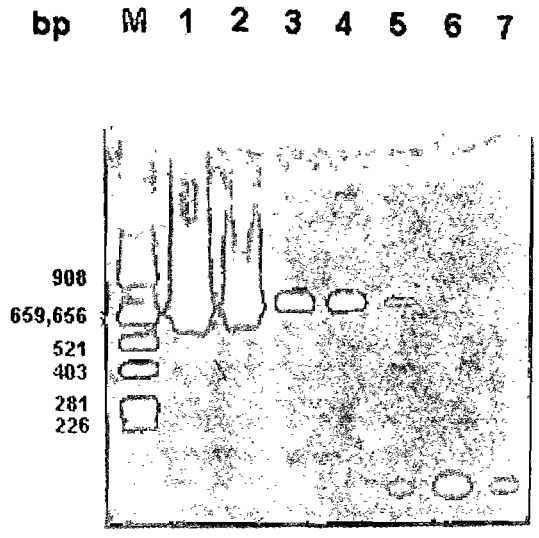

Fig. 2. Sensitivity of PCR assay using different dilutions of template DNA. Lane M: Marker (pBR 322 DNA digested with *Alu* I enzyme), Lane 1 : 100 ng template DNA, Lane 2 : 10 ng template DNA, Lane 3 : 1ng template DNA, Lane 4 . 100 pg template DNA, Lane 5 : 10 pg template DNA, Lane 6 : 1 pg template DNA, Lane 7 : 100 fg template DNA.

These results are in agreement with the findings of Kuppeveld *et al.* (1992), who designed these primers based on 16S rRNA sequence for detection of mycoplasma, acholeplasma, spiroplasma and ureaplasma.The present findings also revealed that this PCR assay can be very well utilised for detection of mycoplasma from field materials which are normally contaminated with bacteria and fungi. Further, it will also be helpful in primary screening of mycoplasma in various infections including the cell cultures, which are very prone to mycoplasma and acholeplasma infections.

The sensitivity of the PCR assay was nearly 10 pg of template DNA (Fig. 2). It indicated that this assay is very sensitive and can be used in case of chronic and subclinical conditions, where very less number of mycoplasmal organisms are present.

Polymerase chain reaction assay was used in the present study for detection of various species of mycoplasma and acholeplasma by using mycoplasma group specific primers GPO-1 and MGSO based on 16S rRNA gene yielded 715 bp amplified product only in the strains of mycoplasma and acholeplasma without any cross-amplification with any other bacterial and fungal strains.

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Haemato-biochemical changes in aflatoxin fed broiler chicks infected with hydropericardium syndrome

S B SHIVACHANDRA[1], R L SAH[2], S D SINGH[3], J M KATARIA[4],T S JOHRI[5] and K MANIMARAN[6]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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Aflatoxin and fowl adenovirus serotype-4, the causative agent of hydropericardium syndrome (Leechi disease), cause anaemia with alterations of serum biochemical changes in broiler chicks (Fernandez *et al.* 1995, Bhatti *et al.* 1989). This communication reports the haemato-biochemical changes in aflatoxin-fed broiler chicks induced with the FAV serotype-4.

The fowl adenovirus serotype-4 (IBH-383/AD/97) maintained in the Division of Avian Diseases, IVRI, Izatnagar, was used for inducing hydropericardium syndrome. Unvaccinated day-old broiler chicks (240), negative for antibodies against FAV serotype-4 and IBD virus, were divided into groups (A, V, AV and C) of 60 birds each.

Group A chicks were fed with aflatoxin diet at 1 ppm level in the feed from day 7 to week 7. Group V chicks were infected intraperitonially at 14 days of age with 0.2 ml of HPS virus (FAV serotype-4) having a titre of $10^{5.5}$ $TCID_{50}$ / 0.2 ml. The chicks in the combined group AV were given both aflatoxin and infected with HPS virus at 14 days of age. Group C served as control. The chicks of all groups were given feed and water *ad lib.* All the chicks were observed daily for clinical signs and mortality up to 5 weeks post inoculation.

Blood samples were collected at weekly intervals from all groups of birds. The EDTA admixed blood (1-2 mg/ml) was used for haematological values using standard methods. MCV, MCH and MCHC were calculated from the pooled data for Hb, TEC and PCV in each group. The sera were separated from blood samples and analysed for serum enzymes using diagnostic kits. SGOT and SGPT levels were estimated according to the 2,4-DNPH method. Serum alkaline phophatase activity, total serum proteins, albumin and globulins were also estimated using standard methods. The data were analysed statistically (Snedecor and Cochran 1967).

Present address: [1,6]Ph.D. Scholars, Division of Bacteriology and Mycology; [2]Principal Scientist, [3,4]Senior Scientist, Division of Avian Diseases.

[5]Principal Scientist, CARI, Izatnagar 243122.

The clinical signs like dullness, depression and reduced feed intake were noticed in chicks belonging to groups A and V, whereas, high mortality rate (56.66%), and more pronounced clinical signs with greenish-white-mucoid diarrhoea were observed in combined group AV.

Haematological studies revealed a significant ($P<0.01$) reduction in Hb (8.12±0.18 g/dl), PCV (24.37±0.88%), TEC (1.60±0.50 × 10^6/µl), TLC (17.96±0.62 × 10^3/µl) values during first week PI, in group V and progressively lowered values throughout the aflatoxin feeding period in group A chicks. Anaemia noticed in groups A and V are in accordance with reports by Mani *et al.* (1993) in aflatoxicosis and Niazi *et al.* (1989) in HPS affected chicks. In combined group AV a significant ($P<0.01$) reduction in Hb (7.57±0.15 gm/dl), PCV (23.35±1.25%), TEC (1.36±0.42 × 10^6/µl) and TLC (13.52±0.28 × 10^3/µl) were recorded in the first week PI compared to individual groups (A, V and C). Higher MCV values (171.69 fl and 144.21 fl) up to second week PI indicated macrocytic anaemia followed by microcytic anaemia at later stages. Similar changes in haematological values were reported by Sandhu *et al.* (1998) in ochratoxin-fed and IBH virus infected chicken.

Biochemical estimations in group V indicated a significant ($P<0.01$) increase in the levels of SGPT (183.50±4.35 units/ml), SGOT (20.75±1.10 units/ml) and alkaline phosphatase activity (214.30±10.19 KA units) during third week PI, whereas, total proteins (3.24±0.04 g/100 ml), albumin (1.12±0.03 g/100 ml) and globulins (2.11±0.20 g/100 ml) levels were decreased in first week PI. A progressive reduction in total protein, albumin, globulin and increased values of SGPT, SGOT, alkaline phosphatase were noticed in aflatoxin-fed chicks (group A). Similar findings were reported by Brown and Abram (1965) in aflatoxicosis and Iqbal *et al.* (1994) in HPS affected broilers. Serum enzymatic evaluations in combined group AV revealed a highly significant ($P<0.01$) rise in first week PI with the maximum values of SGPT (207.50±4.18 units/ml), SGOT (40.75±2.72 units/ml) and alkaline phosphatase (256.00±9.20 KA units) recorded at later stages of experiment, whereas, total protein (2.48±0.08 g/

100 ml), albumin (1.55±0.02 g/100 ml) and globulin (1.93±0.24 g/100 ml) levels were decreased significantly up to fifth week PI compared to individual groups (A, V and C). These findings are in accordance with the observations made by Sandhu *et al.* (1998).

It could be inferred that severe anaemia and a significant biochemical alterations in combined group AV attributed to FAV serotype-4, was further aggravated by the cumulative depressive effect on haematopoietic system and extensive liver damage by aflatoxin up to 7 weeks of age.

SUMMARY

The present study was conducted to understand the importance of association between widespread presence of avian adenovirus infections and natural occurrence of aflatoxin in the feed. It revealed that the cumulative depressive effect on haematopoietic system leading to severe anaemia in aflatoxin fed broiler chicks infected with FAV-4, render them susceptible to many other infectious agents.

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Comparative morphology of *Sarcocystis suihominis* and *S. miescheriana* in domestic pigs

RUPINDERJIT SINGH AVAPAL[1], J K SHARMA[2] and P D JUYAL[3]

Punjab Agricultural University, Ludhiana, Punjab 141 004 India

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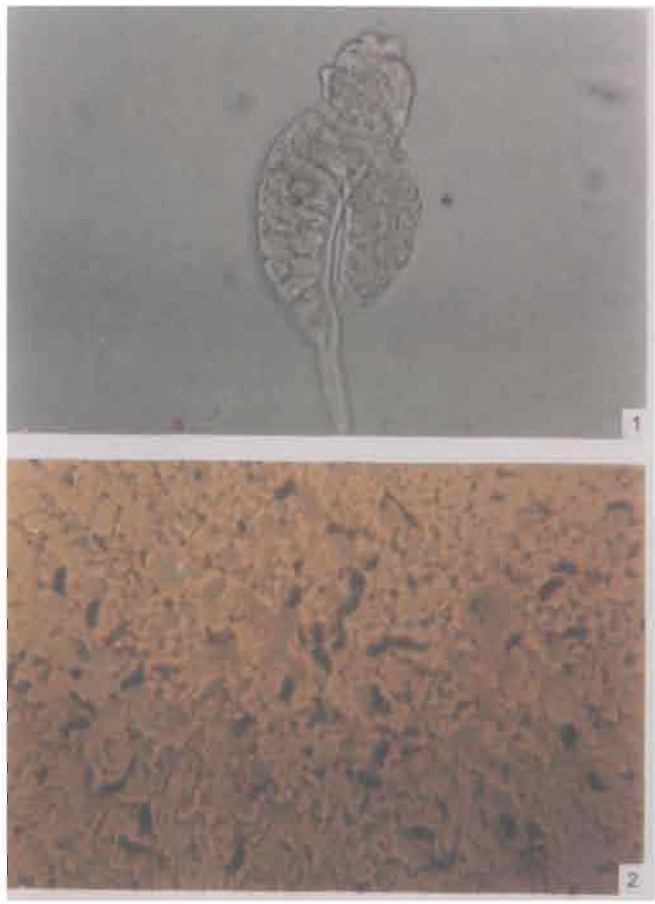
Key words: Morphology, *Sarcocystis*, Pigs

Sarcocysts vary in size and shape, depending on the species of the parasite. The pigs, harbour 3 species of *Sarcocystis*, utilizing different species of definitive hosts. *Sarcocystis miescheriana* has the dog, *S. porcifelis* the cat and *S. suihominis* the man as their respective definitive hosts (Levine 1986). The sarcocysts of *S. suihominis* and *S. miescheriana* are differentiated on the basis of their cyst wall (Dubey and Fayer 1983). *Sarcocystis* infection from the musculature of pigs had been reported in India by a number of workers (Alwar 1958, Agnihotri *et al.* 1987, Solanki *et al.* 1991 and Hemaprasanth and Bhatia 1996). The present communication reports the morphological features of different species occurring in pigs in Punjab state.

A total of 229 animals muscle tissues from thigh, cardiac, jaw, tail, diaphragm, neck, eye muscles, oesophagus and brain collected in polythene bags on ice from various abattoirs in Ludhiana, were immediately brought to the laboratory within 1 hr. The muscle tissues were first examined, grossly. The muscle tissues were separated from the fat and connective tissue as much as possible. Samples were teased with needle in normal saline (0.85%) in watch glass and allowed to stand for sometime. Then examined under stereoscopic microscope and the cysts were isolated as per method described by Juyal *et al.* (1989). These cysts were then transferred to glass slides and examined under low (100×) and high magnification (400×) of microscope after putting a coverslip over it.

The isolated sarcocysts on glass slides were subjected to morphological studies. The microscope was standardized by using stage and ocular micrometer and measurements were taken in the manner described by Sloss *et al.* (1994). Giemsa stain was used as a vital stain. Some of the sarcocysts were ruptured by applying gentle pressure on the coverslip to release metrocytes and bradyzoites which were also examined in fresh and stained preparations.

In this study, 2 types of sarcocysts were collected and identified as *S. suihominis* of public health significance and *S. miescheriana* of dog-pig cycle. Sarcocysts of *S. suihominis* were elongate with both ends tapering. Sarcocysts (n=143) measured 505 to 1605 by 130 to 403 µm with a mean of 893.59 by 239.78 µm. Sarcocysts wall was comparatively thicker than *S. miescheriana* having hair like, straight projections on outer surface, showing tendency to fold on themselves as



Figs 1-2. **1.** Cyst wall of *Sarcocystis suihominis* showing hair like projections (arrow, 140×). **2.** Giemsa stained banana shape bradyzoites (arrow, 450×).

Present address: [1]Ph.D. Fellow, [2]Professor-cum-Head, Veterinary Public Health; [3]Professor, Veterinary Parasitology.

shown in Fig. 1. The hair like protrusions (n=21) measured between 8.9 to 17.4 µm with a mean of 14.18 µm. Sarcocyst cavity was divided into a number of compartments by septa originating from cyst wall. Metrocytes (n=67) were measured and ranged between 7.16 to 13.00 by 3.44 to 7.08 µm with a mean of 9.79 by 4.93 µm. Bradyzoites were measured and size ranged between 17.37 to 21.60 by 2.49 by 4.32 µm with a mean of 18.95 by 3.33 µm.

The morphology of the *S. suihominis* is in general to the description of the sarcocysts as mentioned by Solanki *et al.* (1991) with some minor variations. Solanki *et al.* (1991) described the wall of *Sarcocystis suihominis* as having hair like protrusions, which were confirmed here.

The size of the cyst (n=99) of *S. miescheriana* varied from 428 to 1398 µm by 101 to 292 µm with a mean of 850.73 by 184.44 µm. Sarcocysts were elongate, spindle shaped with both ends tapering. In case of cyst wall, there were papillomatous projections (radially striated or perpendicular protrusions) on outer surface. These papillomatous projections (n=21) were ranged between 2.8 to 5.6 by 1.2 by 1.9 µm with a mean of 4.28 by 1.67 µm. Sarcocyst cavity was divided into a number of compartments by septa which were prominent on upper size range sarcocysts and originate from cyst wall. They were eosinophilic, when stained with H & E stain. Immature sarcocysts contained metrocytes in large numbers with few bradyzoites. Metrocytes (n=49) were either oval or suboval; their size varied from 8.9 to 12.69 by 3.74 to 5.51 µm with a mean of 10.88 by 4.58 µm. Mature sarcocysts contained only bradyzoites. These were in 2 forms, 1 having banana shape with 1 end broad and other rounded and second having slender and crescentric form with 1 end pointed sharply as shown in Fig. 2. Bradyzoites (n=50) were measured with a range of 15.6 to 21.6 by 2.36 to 3.43 µm with a mean of 17.48 by 2.72 µm. The description of the sarcocysts of *S. miescheriana* were similar to Solanki *et al.* (1991) except for minor variations. However, the sarcocysts of *S. miescheriana* differed here from the descriptions of the sarcocysts of *S. miescheriana* by Agnihotri *et al.* (1987) who made no mention of papillomatous projections. It is to point here that the species were identified on the basis of morphology of its sarcocysts, but not on the basis of tissue feeding to their definitive hosts. It is also to be mentioned out here that the primary cyst wall was a reliable criteria rather than the size and shape of metrocytes and bradyzoites.

SUMMARY

Immature and mature sarcocysts of *Sarcocystis miescheriana* and *S. suihominis* were isolated. The size of cysts (n=99) of *S. miescheriana* varied from 428 to 1 398 µm by 101 to 292 µm with a mean of 850.73 by 184.44 µm, while the size of cysts (n=143) of *S. suihominis* varied from 505-1 605 by 130-403 µm with a mean of 893.59 × 239.78 µm. The primary cyst wall of sarcocysts of *S. suihominis* had hair like projections. Morphological description of metrocytes and bradyzoites have also been discussed.

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Prevalence of gastro-intestinal helminths in equines in some hilly pockets of western Himalayas

P P SENGUPTA[1] and M P YADAV[2]

National Research Centre on Equines, Sirsa Road, Hisar, Haryana 125 001 India

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Key words: Donkeys, Egg per gram (EPG), Faecal sample, Horses, Mules, Parasites

The remote areas of this part of Himalayas are not connected by roads and hence not approachable by automobiles. Therefore the equines have a very important role in these areas in carrying the loads. The parasitic diseases are responsible for the enormous losses in droughtability of the animals. Some times apparently animals remain healthy but harbour a heavy parasitic loads and act as the source of the infection to other healthy animals. In recent past, there were a few reports on the incidence of parasitic infections in equines in planes of India (Chaudhri *et al.* 1985, Sengupta and Yadav 1997, 1998 and 2001) but there is no report of parasitic incidence of equines in hilly region of India. In the present study for the first time the amplitude of gastro-intestinal parasitic infections qualitatively and quantitatively has been measured systematically in equines in some hilly pockets of Western Himalayas situated in Himachal Pradesh, Uttaranchal and Jammu and Kashmir. The animals incorporated in the present study did not undergo any deworming schedule. The other aim of the present study was to generate a base line information of the natural parasitic fauna in the equines of this region who were apparently healthy and did not receive any deworming treatment and maintained mainly on natural pasture grazing. These animals were used in carrying loads in hilly tracts.

In the present study the samples were collected from horses, mules and donkeys from Gopalpur, Yol, Kanked, Palampur, Shila, Pathar, Pulani, Baijnath and Kinnartu areas of Himachal Pradesh; Rishikesh area of Uttaranchal and Katra area of Jammu and Kashmir. These animals were maintained on grazing and no good management care including balanced ration was given to the animals. They did not undergo any deworming schedule. A population of 139 equines comprising 86 horses, 18 donkeys and 35 mules were incorporated in the present study. The faecal samples were collected either per rectum or fresh at defaecation. The faecal samples were collected in airtight containers. For determination of worm load eggs per gram (EPG) of faeces was calculated by Mc Master counting chamber. For each animal, 3 observations were made and the mean counts were calculated and recorded as the count.

For identification of larval stages, faecal samples positive for strongyles, were kept under incubation at 27°C for 7-10

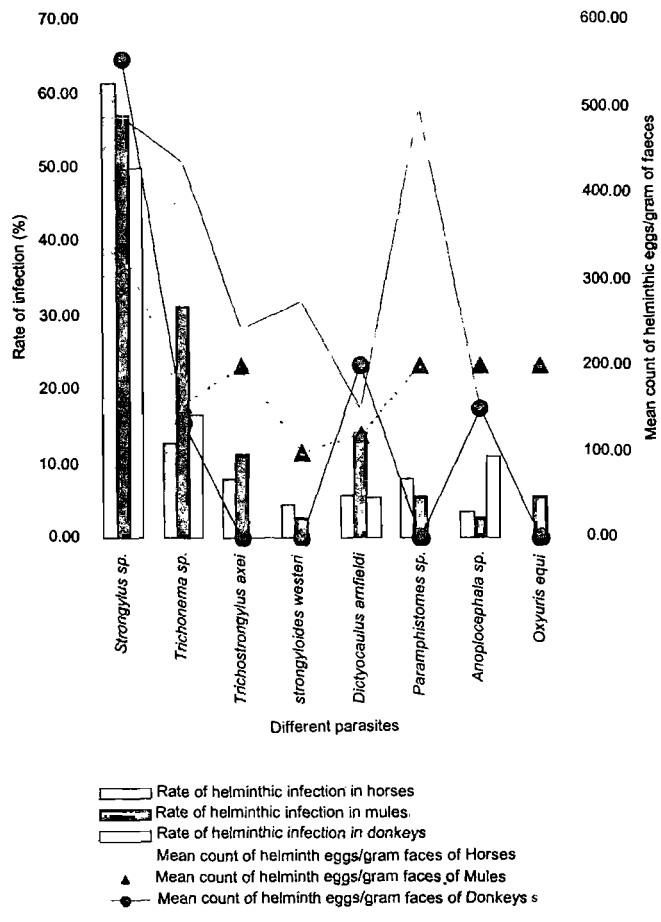

Fig. 1. Pattern of prevalence of gastro-intestinal helminths in equines.

Present address: [1]Scientist (S.S.), PD-ADMAS, Hebbal, Bangalore 560 024

[2]Director, IVRI, Izatnagar, Uttar Pradesh 243 122

days and the recovered third stage larva were identified. Identification of larva and assessment of worm load (EPG) were made as per Soulsby (1965) and Anonymous (1971). The statistical calculation was done as per Snedecor and Cochran (1968).

Fig. 1 is showing the pattern of prevalence of gastro-intestinal helminths in equines (horses, donkeys and mules). *Strongylus* spp. infection showed major gastrointestinal parasitic infection in horses, donkeys and mules recording the rate of infection, respectively, 61.62%, 50.00% and 57.14%. The mean EPG was found as 488.37, 555.55 and 340.00, respectively, in horses, donkeys and mules. In other study at different parts of the country *Strongylus* spp. infection was recorded as major infection in equines and the species of larva were also recorded (Chaudhri *et al.* 1985, Sengupta and Yadav 1997 and 1998). The average EPG found in horses in the present study falls between the findings of Chaudhri *et al.* (1985) and Sengupta and Yadav (1998). *Trichonema* spp. infection was the second highest gastrointestinal parasite recorded in the present study with rate of infection 12.79%, 16.66% and 31.42%, respectively, among horses, donkeys and mules. This infection was also observed by Rai and Srivastava (1958) in donkeys and Thapar (1957) by necropsy findings. Chaudhri *et al.* (1985) recorded 20.4% infection rate in donkeys and Sengupta and Yadav (1997) recorded 15.63% in equines in Haryana. In the present study. The mean EPG was 436.36. In previous studies in the planes of Uttar Pradesh and Haryana mean EPG with a range from 200.00 to 555.00 have been recorded (Sengupta and Yadav 1997, 1998). *Trichostrongylus axei* infection was observed in mules (11.42%) and horses (8.13%). Chaudhri *et al.* (1985) and Sengupta and Yadav (1997) recorded 3% and 6.25%, respectively, among equines in Haryana. The mean EPG in this study was 242.85 to 200.00. In earlier study in planes of Uttar Pradesh and Haryana, it was 425.00 to 180.00. *Strongyloides westeri* infection was recorded as 4.65% in horse and 2.85% in mules, which is lower than the rate found by Chaudhri *et al.* (1985). This sp. was also recorded by Pande and Rai (1960) in donkey foals. *Dictyocaulus arnfieldi*, the causative agent of verminous pneumonia in equines were recorded in mules (14.28%), horses (5.81%) and donkeys (5.55%). In the present study, the rate of infection is more than the previous study as 1.61% in planes of Uttar Pradesh (Sengupta and Yadav 1997). This is probably due to much more favourable atmospheric condition i.e. humid low temperature in hilly areas. *Oxyuris equi*, the pin-worm, was recorded from 5.71% in mules. In an earlier study 1.81% infection rate was recorded in horses in plane of Uttar Pradesh (Sengupta and Yadav 1997). After coproculture *S. edentatus*, *S. equinus*, *S. vulgaris*, *Triodontophorus* spp. *Oesophagodontus* spp. and *Gyalocephalus* spp. larva were recovered @ 56.60%, 52.83%, 52.83%, 35.84%, 16.98%, 9.43%, and 7.54%, respectively, in horses; 60.00%, 45.00%, 50.00%, 10.00%, 5.00% and 15.00%, respectively, in mules and 55.55%, 55.55%, 44.44%, 22.22%, 11.11% and 11.11%, respectively, in donkeys. In an earlier study in plane of Uttar Pradesh *S. equinus*, *S. edentatus* and *S. vulgaris* were recorded from equines up to 50.00%, 45.00% and 26.08%, respectively, (Sengupta and Yadav 1997). In the present study *Paramphistomes* spp. were recorded 8.13% in horses and 5.71% in mules. Chaudhri *et al.* (1985) recorded 1.8% infection rate in Haryana in equines. Damodaran *et al.* (1978) recorded 0.24% in equines in Madras. *Anoplocephala* spp. infection was found 11.11%, 3.48% and 2.85%, respectively, in donkeys, horses and mules. Sengupta and Yadav (1998) recorded 2.12% infection rate in equines from Haryana.

In the present study, the major gastro-intestinal parasite was found as *Strongylus* spp. in horses, donkeys and mules. There is a low infection rate in animals rate in animals and they are apparently healthy. Due to low grade infection the animals are probably preimmune and the infection rate and EPG is restricted to a certain level due to host immune mechanism. But this host-parasite balance may disturb if the animals go through any stress or immuno suppression process and parasites may come on upper hand. Therefore the animals are at the risk of colic, formation of nodules in large intestines, aneurysms in the mesenteric arteries, ulcers in the stomach and intestines, pneumonia, anorexia, prolapse of rectum etc. (Sastry 1983, Soulsby 1986 and Latasana Devi *et al.* 1996). Further detail statistical study may reveal economic losses and risk analysis. Animals should go through a routine deworming schedule.

SUMMARY

A study to measure the incidence of gastro-intestinal parasitic infections qualitatively and quantitatively in apparently healthy non-treated equines in hilly tracts of western Himalayas (Himachal Pradesh, Uttaranchal and Jammu and Kashmir) were carried out to generate a baseline data of the natural parasitic fauna in equines in that region. *Strongylus* spp. infection was predominantly present in horses (61.62%), donkeys (50.00%), and mules (47.14%). *Strongylus equinus*, *S. edentatus*, *S. vulgaris*, *Trichonema* spp., *Trichostrongylus axei*, *Dictyocaulus arnfieldi*, *Oxyuris equi*, *Strongyloides westeri*, *Oesophagodontus* spp., *Triodontophorus* spp., *Gyalocephalus* spp., *Anoplocephala* spp. and *Param-phistomes* spp., were also recorded.

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Parasitic infections among monkeys in Tamil Nadu

G PONNUDURAI[1], T ANNA[2] and T J HARIKRISHNAN[3]

Tamil Nadu Veterinary and Animal Science University, Namakkal, Tamil Nadu 637 001 India

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Key words: Monkey, Parasitic infection

Thousands of monkeys are found in the District Livestock Farm, Pudukkottai, Tamil Nadu, whose parasitic spectrum is not known. Hence this work was under taken to study the parasitic infection among monkeys.

The study was undertaken at district livestock farm, Pudukkottai. Faecal samples (108) were collected separately in polythene bags for examination. Positive samples were subjected to faecal culture for specific identification of helminth parasite based on larval morphology and morphometry. The larvae harvested from faecal culture were stained with Lugol's iodine for detailed study.

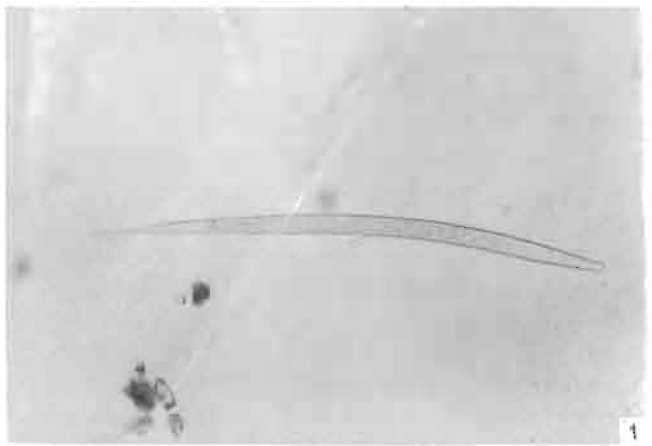
Samples (56) were found positive for parasitic infection giving an over all percentage of 51%, in this study. The morphological characters of parasite encountered in this study were in accordance with Soulsby (1987) and were identified as *Entamoeba* spp., *Oesophagostomum* spp. and *Strongyloides* spp (Figs 1,2).

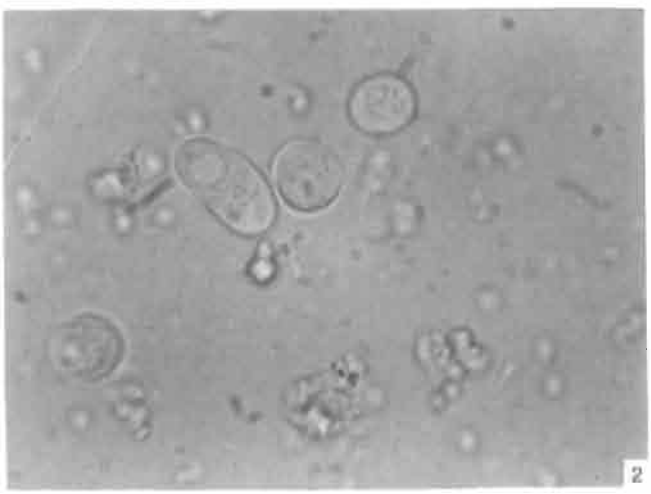
The prevalence of amoebiasis in monkeys has been reported by Sano *et al.* (1980), Pang *et al.* (1993) and by Ravi Kumar and Hafeez (1995) in India who observed the prevalence to be 14.47% in captive monkey of Andhra Pradesh. Samanta and Dey (2000) recorded parasitic prevalence as 60.97% in summer, 82.60% during rainy and 53.06% in winter season. Whereas in the present investigation the prevalence of *Entamoeba* spp. was 14%. The prevalence rate of 14% in this study in high considering health hazard to humans, since amoebiasis is primarily a disease of humans (zooanthroponosis).

Soulsby (187) and David (1986) had earlier reported the occurrence of *Oesophagostomum* spp. and *Strongyloides* spp. in monkeys. In the present study *Oesophagostomum* spp. and *Strongyloides* spp. accounted for 29 and 14%, respectively, and prevalence of mixed infection was 7% in this study.

SUMMARY

Faecal samples (108) of monkeys were examined between August 2000 and February 2001 for intestinal parasitic infection. Of these 56 (51%) samples were positive for parasitic infection. Among the parasitic infections, *Entamoeba* spp. accounted for 14%, *Oesophagostomum* spp. 28% and *Strongyloides* spp. 14%. The incidence of 3 parasites was seen as mixed infections in 7%.





Figs 1-2. **1**. Larval stages of *Oesophagostomum* spp. **2.** Various stages of *Entamoemba* spp.

Present address: [1,2]Assistant Professor, [3]Associate Professor and Head, Department of Veterinary Parasitology, Veterinary College and Research Institute.

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Evaluation of different internal fixation techniques for the management of comminuted femoral fracture in dog: A scanning electron microscopic (SEM) study

B SARAVANAN[1], S K MAITI[2], M HOQUE[3], NAVEEN KUMAR[4] and G R SINGH[5]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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Key words: Bone fracture, Dog, Femur, Internal fixation, Scanning, Surgery

Comminuted femoral fractures often a challenging task for orthopaedic surgeons because fragmentation does not lend itself to anatomic reduction and therefore, cannot support the load associated with the ability to bear weight after fixation, causing maximal stress on implants. Additionally, soft tissues are often severely traumatized, reducing vascularity to the fractured area (Hulse and Johnson 1997). Finally compromised soft tissus and lengthy operating time may predispose these fractures to substantial bacterial contamination and infection (Stevenson *et al.* 1986). Rebuliding of comminuted fractures so that all fragments are accurately aligned and gaps are eliminated is often impossible. Non union and osteomyelitis have their highest incidence in these fractures (Smith *et al.* 1978). Over the years, several techniques were tried to treat comminuted femoral fractures with different success rate and there was a lack of uniform fracture presentation. Hence, it would be difficult to compare different techniques in a particular type of fracture. Keeping these facts in view, the present study was therefore, undertaken to evaluate and compare the efficacy of three different internal fixation techniques in the management of comminuted diaphyseal femoral fractures in experimental dogs.

The study was conducted in 15 clinically healthy adult mongrel dogs of either sex which were randomly divided into 3 equal groups (A, B and C) of 5 animals each. In all the animals, unilateral comminuted diaphyseal femoral fracture was created under general (Thiopental – 5%) anaesthesia and immobilized with intramedulllary pinning with cerclage wiring (group A), dynamic compression bone plating (group B) and dynamic compression bone plating with cerclage wiring (group C) respectively. All the animals were administered broad-spectrum antibiotic (streptopenicillin @ 1 g intramuscularly/dog/day) and analgesic (5 ml I/M daily) and surgical wounds were dressed daily with povidone iodine and loraxane cream for 5 days postoperatively.

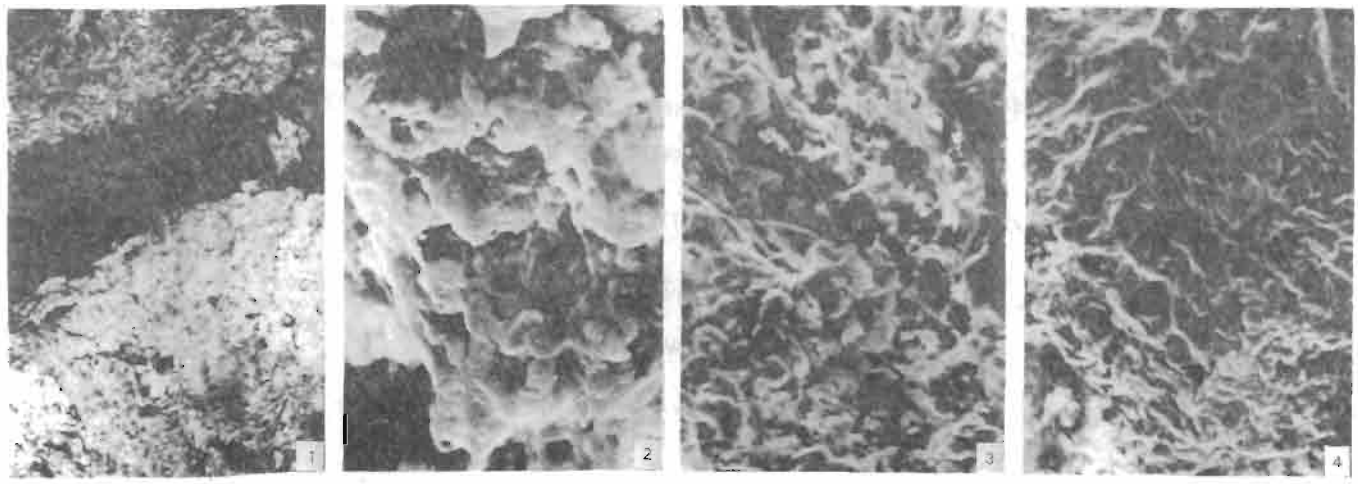
The bone specimens consisting of healed test bone and adjacent host bone, were collected immediately after euthanising the animals on day 60. The specimens were processed as per the standard procedure for scanning electron microscopy.

The scanning electron microscopic (SEM) examination has been used to study the structural aspects of adult and developing bone (Singh *et al.* 2000). This study provides topographic picture of healing site and it became a valuable parameter for evaluating fracture healing study.

In 3 animals of group A, SEM clearly indicated fracture gap as the end of observation period (Fig. 1). In other animals, the gap was partially filled by newly proliferated tissue, which was a mixture of osseous, cartilaginous and fibrous tissue. The osseous tissue was predominantly along the fracture ends, whereas collagenous fibre network was present at the center of the defect and overall, the process of ossification was relatively slow. The gap seen in the SEM section was due to the rotational instability at the fracture site, as IM pinning with wiring unable to prevent the rotational motion at the defect site completely leading to delayed vascularisation and fracture healing (Hinko and Rhinelander 1975, Jones 1994, Rouse and McLaughlin 1998). Intramedulllary fixation should be avoided in comminuted fracture in dogs, as the pin provides no longitudinal support or resistance to shortening forces and so collapse, rotation and non- union are to be expected (Denny 1991). In 2 animals of this group, fracture site was completely filled with fibrous tissue, with little or no signs of ossification indicating non-union. Cerclage appliance generally causes non-union when used simultaneously with IM pin fixation. In most cases, non-union results from 2 problems: (i) the intramedulllary pin and wires, apparently stable at surgery, were inadequate and motion results, (ii) the cerclage appliance impaired or 'strangle' longitudinal bone circulation (Newton and Hohn 1974).

In animals of group B, the SEM picture showed that the fracture gap was completely filled by newly formed osseous tissue / osteon (Fig. 2). At certain areas, the collagenous network was relatively more and newly formed bone appeared more compact, whereas in other areas, the osseous callus were

Present address: [1]M.V.Sc. Scholar, [2,3]Scientist (Senior Scale), [4]Senior scientist, [5]Head, Division of Surgery.



Figs 1-4. **1.** SEM photograph of undecalcified bone section revealing the presence of gap between the fracture ends (100x, day 60, group A). **2.** SEM photograph of undecalcified bone section showing characteristic osteon formation with large interspace between them (5000x, day 60, group B). **3.** SEM photograph of undecalcified bone section at fracture site depicting newly formed vascular network and bone matrix in the process of calcification (5000x, day 60, group B). **4.** SEM photograph of undecalcified bone section depicting fibrocartilagenous reaction partially filled up the fracture defect (5000x, day 60, group C).

scattered but there were indication of vascularization and laying down of bone matrix. Further magnification showed the presence of newly formed blood vessel network and bone matrix, which was in the process of calcification (Fig. 3). Bone plating with interfragmentary compression screws (neutralization plating) provides the most stable reduction and is especially indicated in comminuted femoral diaphyseal fracture (Milton and Newman 1985). This neutralization plate provided rigid fixation in these animals as also reported by other workers (Denny 1991, McLaughlin and Roush 1999) and showed better healing pattern than IM pinning with full cerclage wiring.

In group C, in which comminuted fracture fragments were immobilized with bone plating and full cerclage wiring showed mixed results. In 3 animals, where fixation devices remained intact up to the end of observation, fracture gap almost completely filled with newly formed osseous tissue. Endochondral ossification was seen with newly formed osteon replacing the old cartilage. Prominent osteoblastic and osteoclastic activity was evidence at places. In between these cells, budding blood vessels and collagen network were also evident. These blood vessels originate mainly from medullary and periosteal vessels and also from surrounding soft tissues. In 2 animals of this group, delayed union was evident with fibrous tissue filling the fracture gap. However, a fibrocellular reaction near the periosteum was suggestive of some degree of bone healing by cartilage formation (Fig. 4). These results suggested that full cerclage wiring with compression bone plating did not provide expected better results than bone plating when used alone (group B). Fixation of bone fragments by cerclage wiring had a deleterious effect on cortical blood circulation, subsequent bone healing and callus bridging (Newton and Hohn 1974). The other inherent danger of cerclage wiring was that the wire might become loose, break or slippage into the fracture line causing fracture instability. The wire would then rub against the fracture fragments producing an area of devitalisation by preventing the invasion of small blood vessels, which lead into delayed/non-union (Jones 1994), which was also observed in this study.

SUMMARY

Efficacy of three different internal fixation techniques in the management of comminuted diaphyseal femoral fractures in dogs, was evaluated. The full cerclage wiring with compression bone was not better than bone plating when used alone.

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Development of a simple and modified Thygesen's fetatome for field applications

JOHN ABRAHAM[1] and R P VERMA[2]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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Key words: Fetatome, Fetotomy, Obstetrical technique

Fetotomy is one of the obstetrical techniques routinely being used by field veterinarians in difficult obstetrical cases like dead and emphysematous foetus to avoid the risk of stress and injury caused by excessive traction and manipulation (Roberts 1971). The fertility of the dam is less likely to be impaired by this technique and the recovery from fetotomy takes less time than from caesarian section (Arthur *et al.* 1982). Several difficulties are encountered with the conventional Thygesen's fetatome consisting of parallel metal tubes with hardened steel heads through which a braided steel cutting wire is looped using a fetatome threader (Sloss and Duffy 1980). The conventional instrument is very heavy and not easy to handle and operate. Threading of the conventional fetatome is a cumbersome process, as the eye of the threader breaks easily and the wire slips and gets stuck in the barrel. The kinking of the saw-wire inside the barrel and the difficulty to clean and sterilise the instrument are also the drawbacks of the conventional instrument.

A modification of this instrument was attempted to overcome these problems and to make it simple and easy to handle. The main aim of the modification was to eliminate the use of fetatome threader, which causes much trouble during the operation, and to make the instrument lighter for easy handling and maneuverability. These were accomplished by designing a double grooved locking system made up of aluminium pipes. The modified instrument (Fig.1) is also a double barrel or guard type. Inside each barrel is fixed 1 more barrel of slightly less diameter so that it can rotate freely within the first one (Fig.2).

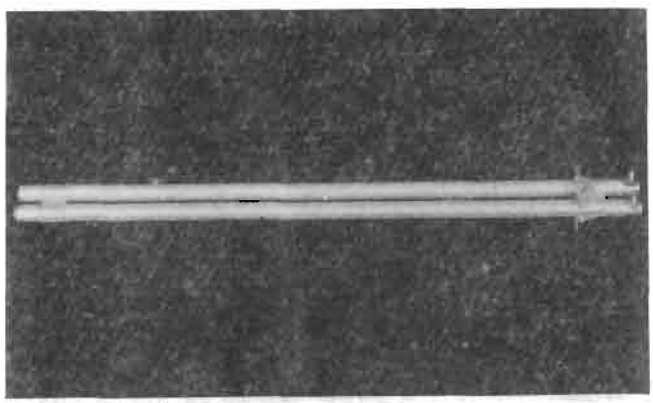

Fig.1. Modified Thygesen's fetatome.

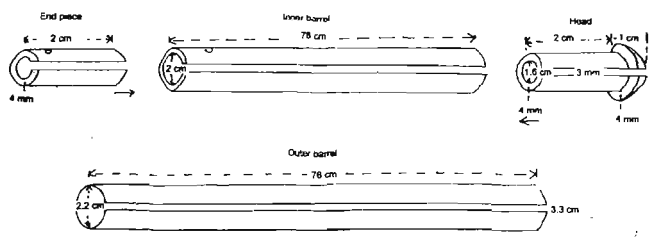

Fig.2. Parts of the inner and outer barrel.

The head of the modified fetatome is made up of a brass cylinder, 2-cm long and 4-mm thick. The head has a 2-mm projection at the brim and the anterior end is smooth and rounded. A 3-mm groove is made on the lateral side and it is fitted on to the inner aluminium barrel, which also has a groove of 3-mm running through its entire length (Fig.2). The length of the inner barrel is 78 cm and its diameter is 2 cm. The head is fitted on to it, taking care that the grooves coincide. The other end of this barrel is attached with a brass cylinder, 2 cm long and 4 mm thick with a 3-mm lateral groove. This is to prevent the cutting away of the edges by the saw-wire and for fixing the lock handle.

An outer aluminium barrel (76 cm long, 2.2 cm diameter and 2 mm thick) which also has a 3 mm groove on its lateral side running through its entire length is then introduced over the inner barrel assembly. The handle locks are screwed to the projecting part of the inner barrel through the end piece.

Two such barrels are fixed at a distance of 1-cm using a twin 'C' clamp attachment (2.5 cm broad), 5 cm from the head and 7 cm from the handle (Fig.3). All the ends are grinded to ensure a smooth finish. The chain fixation plate is made up

Present address: [1]Sharon, Santhosh Nagar, Mannuthy, Thrissur, Kerala 680 651.

[2]Principal Scientist, Division of Animal Reproduction.

Fig.3. Completed assembly of barrel No.1.

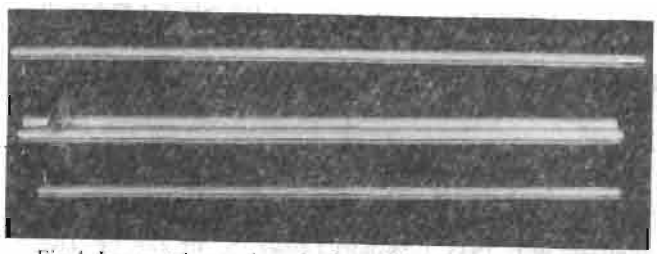

Fig.4. Inner and outer barrel of modified Thygesen's fetatome.

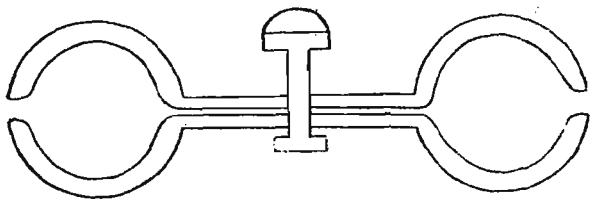

Fig. 5. 'C' clamp attachment.

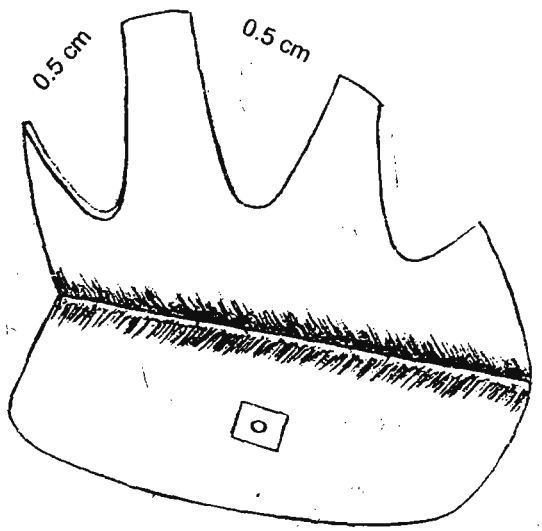

Fig. 6. Chain fixation plate.

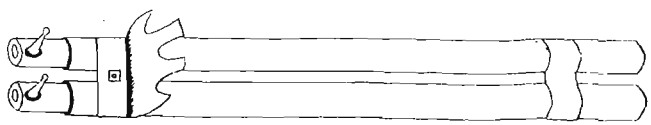

Fig.7. Complete assembly of modified Thygesen's fetatome.

of tempered steel to attach obstetrical chains. It is a semi-circular metallic piece of 3.5 cm radius, one-half bend perpendicularly for attachment with a similar piece on the opposite side, with 'U' shaped notches (3 in number) of 0.5 cm width at the top, tapering gradually towards the bottom (Fig.4).

When the handle locks are held up vertically, the grooves of both the barrels come in the same line and the barrels are open. The wire-saw, which has already been passed over the foetus, can be easily inserted into the fetatome. With a twist of the handle lock at 180°, the barrels close and the handle gets locked into each other. Now the instrument can be positioned conveniently inside the uterus and is ready for operation. When the operation is over, by unscrewing the handle locks, the 2 barrels can be separated and can be easily cleaned and sterilised.

Proper lubrication is a must for the success of the operation (Noakes 1988). A light epidural anaesthetic (7 ml of local anaesthetic solution in heifers and small cows and 10 ml of 2 - 2.5% solution for large cows) may be administered prior to fetotomy to prevent continual straining, sudden movements resulting from pain and to keep the animal standing (Arthur *et al.*1982).

The modified fetatome was under trial at Indian Veterinary Research Institute, where it has been successfully used in cases of dystocia due to foetal death in both cows and buffaloes. The modified instrument has been successfully used in cases of dystocia due to foetal death at the veterinary dispensary, Kopalam, Kerala State.

It is concluded that the modified fetatome has advantages over the conventional fetatome because it is lighter (<0.5 kg) in weight, and is easy to handle and operate; easily threadable and more safe due to it's locking system; eliminates the use of saw-wire threader, which usually creates problem during operational process of conventional fetatome; is conveniently threadable after passing the wire-saw over the foetal part inside the uterus and also threadable even with the wire-saw handle attached; can be easily dismantled, cleaned and sterilised; can be easily duplicated in the field conditions and is made up of locally available cheap aluminium pipes and other materials; and is easy to transport (Table 1).

Table 1. Comparison of the conventional fetatome and modified fetatome

Particulars	Conventional fetatome	Modified fetatome
Material	Hardened steel	Aluminium pipes
Length	90 cm	78 cm
Width	5 cm	5 cm
Distance between the tubes	1 cm	1 cm
Thickness of the tube	1 cm	2 mm
Weight	1.260 kg	0.480 kg

SUMMARY

A simple modified version of the Thygesen's fetatome was designed and fabricated as a recent advancement in obstetrical technique for field application. The improved new model of Thygesen's fetatome was made of aluminium tubes (2mm thick) in which wire-saw threader was replaced by a double grooved lock system. The model is simple to apply during fetotomy process as this is lightweight (<0.5 kg), easy to handle and easily threadable without a threader. Besides, this is inexpensive and could be used even under ordinary field conditions.

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Peripheral plasma inhibin concentrations in relation to expression of estrus in Murrah buffaloes (*Bubalus bubalis*)

S MONDAL[1], B S PRAKASH[2] and P PALTA[3]

National Dairy Research Institute, Karnal, Haryana 132 001 Indta

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Key words: Buffalo, Estrus expression , Inhibin

Poor expression of estrus hampers efficient utilization of tropical buffaloes. Heat detection in buffaloes is very difficult as overt signs are of low intensity and of short duration (Jainudeen and Hafez 1987). Moreover, silent estrus is quite common in buffaloes during the stressful summer as well as the nonstressful winter (Kamboj and Prakash 1993). The behavioural symptoms are induced primarily by the action of estrogen on the central nervous symptom. Inhibin, a glycoprotein hormone shares a number of similarities with estrogens. Both these hormones are produced by granulosa cells of the ovarian follicles under the influence of follicle stimulating hormone (FSH) and both of them are involved in the suppression of hypophysial secretion of FSH through negative feedback (Campbell *et al.* 1990, Taya *et al.* 1996). There is no information available on peripheral plasma inhibin concentrations in relation to expression of behavioural estrus symptoms in buffaloes. The present study was therefore undertaken to measure peripheral inhibin concentrations during estrous cycle and to relate them to occurrence of overt or silent estrus in Murrah buffaloes.

Cyclic non-lactating Murrah buffaloes (n=5) were selected from the National Dairy Research Institute animal herd and maintained under standard feeding and management conditions. Blood samples were collected once daily for consecutive 32 days during January and February. Plasma samples were stored at –20°C until subsequent analysis for inhibin and progesterone concentrations. Estrus was detected by parading a vasectomized bull twice daily and visual symptoms, and subsequently confirmed by plasma progesterone estimation. Inhibin concentrations were determined by a sensitive double antibody RIA using highly purified 32 kDa bovine inhibin for iodination, an antiserum against bovine inhibin (#1989) and purified 31 -kDa bovine inhibin (bINH-R-90/1) as reference preparation, as described previously (Palta *et al.* 1996) was used. The sensitivity of inhibin assay was 16 pg/tube and the intra- and inter-assay coefficients of variation were < 13 %. Progesterone concentrations were determined by a simple, direct RIA described previously (Kamboj and Prakash 1993). The sensitivity of progesterone assay was 8 pg/tube and the intra- and inter-assay coefficients of variation were < 17 %. For statistical analysis, the oestrous cycle was divided into 4 phases viz, late luteal (day - 4 to day - 2, day 0 = day of estrus), periestrus phase (day -1 to day 1), early luteal phase (day 2 to day 5) and midluteal phase (day 6 to day 14). Inhibin concentrations at different phases of the oestrous cycle were compared between animals that exhibited overt estrus and those that had silent estrus by ANOVA.

Out of total 7 estrus, 2 were accompanied by overt signs, whereas rest 5 were silent estrus. Mean (±SEM) inhibin concentrations at different days of estrous cycle in buffaloes which exhibited overt or silent estrus are shown in Fig. 1. When the oestrous cycle was divided into 4 phases (Fig. 2), peripheral plasma inhibin concentrations were significantly

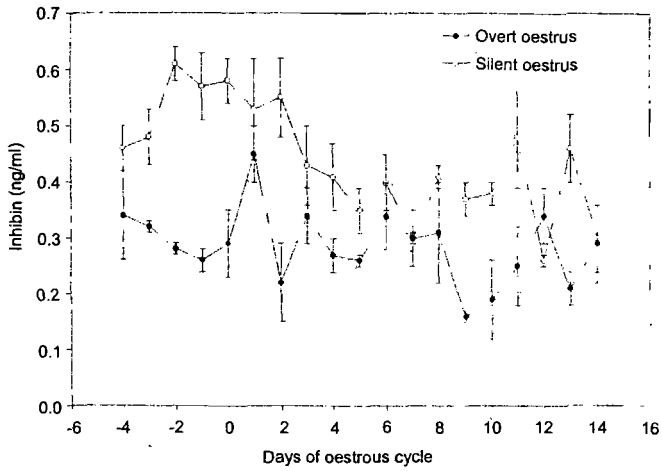

Fig. 1. Mean (±SEM) peripheral plasma inhibin concentrations during different days of the oestrous cycle in buffaloes that exhibited overt or silent oestrus (day 0 = day of oestrus).

Present address: [1]Scientist, National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore 560 030.

[3]Senior Scientist, Animal Biotechnology Centre; [2]Principal Scientist and Head, DCP Division.

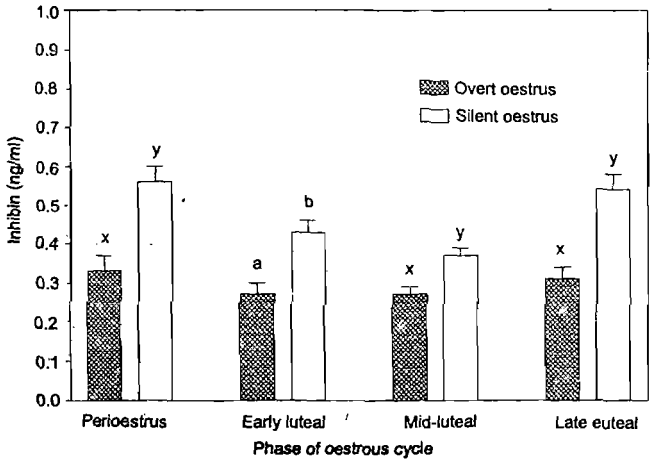

Fig. 2. Mean (±SEM) peripheral plasma inhibin concentrations during different phases of the oestrous cycle in buffaloes that exhibited overt or silent oestrus (*mid-luteal phase*: day 6 to day 14, day 0 = day of oestrus; late luteal phase: day -4 to day -2; perioestrus phase: day -1 to day 1; early luteal phase: day 2 to day 5; Columns with different letters differ significantly a,b: $P<0.05$; x,y: $P<0.01$).

higher in animals exhibiting silent estrus compared to those that exhibited overt signs of estrus at late luteal, periestrus and midluteal phases ($P<0.01$) and at early luteal phase ($P<0.05$)

The present study demonstrates that peripheral plasma inhibin concentrations exhibit a dynamic profile during oestrous cycle and that inhibin concentrations are higher in buffaloes that exhibit silent estrus than those which show overt behavioural signs. During the oestrous cycle, the pattern of peripheral plasma inhibin concentrations was similar irrespective of the nature of estrus expression. Inhibin concentrations which were lowest during midluteal phase increased through the late luteal phase to reach the highest values during the periestrus phase. These results are in agreement with earlier observations in cattle (Kaneko *et al.* 1992) and buffalo (Palta *et al.* 1997) in terms of an increase in inhibin levels during the follicular phase of estrous cycle and a decrease thereafter.

In bufffaloes, the follicular turnover during the oestrous cycle occurs in a distinct wave like pattern, with predominance of a two-wave pattern (Manik *et al.* 1998). The second wave and in case of a 3 wave pattern, the third wave which marks the emergence of the dominant ovulatory follicle begins on days 10 and 19 respectively (day 0 = day of ovulation). The dominant ovulatory follicle is estrogen-active and is expected to secrete increasingly large quantities of estrogen initially under the influence of FSH and subsequently under the influence of LH as it grows to the preovulatory stage (Hillier 1981). The preovulatory follicle is the source of peripheral peak in blood estrogens which precedes the surge of LH (Moor *et al.* 1975). It is possible that the higher concentrations of inhibin during the midluteal, late luteal and periestrus phase as observed in buffaloes which exhibited silent estrus could have resulted in suppression of peripheral FSH concentrations thereby adversely affecting estrogen production by the dominant ovulatory follicle. Low production of estrogens by this follicle, as reached the preovulatory stage could presumably have resulted in the failure of buffaloes to exhibit behavioural signs of estrus.

In conclusion, the results of this study indicate that peripheral plasma inhibin concentrations are higher ($P<0.01$) in buffaloes that exhibited silent estrus than those in overt behavioural signs.

SUMMARY

This study investigated the changes in peripheral plasma inhibin levels in relation to expression of estrus in Murrah buffaloes. Out of total 7 estrus, two were accompanied by overt signs, whereas rest 5 were silent estrus. Inhibin concentrations were lowest during midluteal phase and increased through late luteal phase to reach the highest values during the periestrus phase in buffaloes that exhibited overt signs and silent estrus respectively. It was concluded that inhibin levels were significantly higher ($P<0.01$) in buffaloes that exhibited silent estrus compared to overt estrus and might be responsible for poor expression of estrus.

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Factors affecting postpartum oestrus interval in goats

NEERU[1], DHARM SINGH[2], PUNEET KUMAR[3] and A K GOEL[4]

Central Institute for Research on Goats, Makhdoom, P.O. Farah, Mathura, Uttar Pradesh 281 122 India

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Key words: Goat, Post-partum oestrus, Parity, Season

Reproduction is a key phenomenon for perpetuation of the population. In domestic ruminant species like goat parturitions are not regularly distributed throughout the year. The kidding season in goats was reported from September to March (Maule 1962). Therefore the postpartum oestrus interval in goats is very critical for reproductive performance. Acharya (1988) has reported kidding interval in Indian goat breeds ranging from 282 in Bengal goats to 376 days in Malabari breed. This showed the highest inter-breed variation in reproductive performance of tropical goat breeds. The present study was undertaken to study the effect of breed, parity and season of kidding on postpartum oestrus interval in north-western Indian breeds of goats.

Observations on 183 lactating does of north-western Indian breeds (96 Sirohi, 41 Marwari and 46 Kutchi) maintained at the Central Institute for Research on Goats (CIRG), Makhdoom (latitude 27° 10' N and longitude 78° 02' E) during the year 1992 to 1996 were recorded. The effect of breed, parity and season of kidding was studied. Goats were maintained under semi-intensive system of management with 4-6 hr daily grazing. The concentrate mixture in the form of pelleted feed (DCP 13% and TDN 67%) was offered @ 300g/hr/day during advance pregnancy and 400g/kg of milk during lactation. Cultivated green fodder @ 1kg/hr/day and freshwater was offered twice daily. Kids remained with their dams until 4 days after kidding. Thereafter, they were allowed to suckle their dams in the morning and evening twice daily up to 90 days of age. Goats detected in oestrus by parading aproned buck in the morning and evening hours and were bred naturally.

To study the effect of season the whole year was divided into 5 seasons on the basis of mean daily temperature (MDT)

Table 1. Seasonal variation in postpartum oestrus interval in goats

Season of kidding	Postpartum oestrus interval (days)									
	Sirohi			Marwari			Kutchi			Pooled
	Primiparous	Pluriparous	Pooled	Primiparous	Pluriparous	Pooled	Primiparous	Pluriparous	Pooled	
Comfortable (Oct. and Nov.)	232.4±10.1 (27)	205.1±11.1 (31)	217.8±7.7 (58)	208.8±7.0 (11)	230.1±16.7 (8)	217.8±8.2 (19)	256.7±40.3 (4)	217.6±14.9 (20)	224.1±14.0 (24)	219.3±5.7 (101)
Cool (Dec. and Jan.)	176.1±10.1 (15)	196.0±28.3 (7)	182.4±11.1 (22)	172.0±24.0 (4)	153.4±29.8 (7)	160.2±20.2 (11)	211.7±19.6 (4)	225.2±43.1 (4)	218.4±22.1 (8)	183.5±9.4 (41)
Comfortable (Feb. and March)	410.2±51.1 (4)	161.6±16.5 (5)	272.09±49.2 (9)	99.3±24.6 (3)	461.0±6.0 (2)	244.0±89.6 (5)	328.0±10.0 (6)	168.7±59.2 (4)	264.3±50.5 (10)	263.0±32.1 (24)
Hot dry (April, May and mid June)	124.0±23.5 (4)	120.5±77.5 (2)	122.8±24.9 (6)	62.6±11.9 (5)	52.0±1.3 (1)	60.8±9.9 (6)	38.0±10.0 (2)	275.0±1.3 (1)	117.0±79.2 (3)	96.8±18.6 (15)
Hot humid (Mid June, July, Aug. and Sep)	65.0±1.3 (1)	-	65.1±1.3 (1)	-	-	-	94.0±1.3 (1)	-	94.0±1.3 (1)	79.5±14.5 (2)
Overall	217.8±12.07 (51)	195.0±9.7 (45)	207.1±7.9 (96)	156.3±14.3 (23)	216.0±27.4 (18)	182.5±15.0 (41)	236.0±33.4 (17)	213.9±14.1 (29)	222.1±15.1 (46)	205.2±6.6 (183)

Figures in parentheses are absolute no. of goat.

Present address: [1]Scientist, [3]Senior Scientist (Animal Physiology); [2]Principal Scientist (LPM); [4]Senior Scientist (Animal Reproduction).

and mean vapour pressure (MVP) (Singh *et al.* 1989) as follows. Comfortable (February to March, MDT 19°C and AVP 12.67 mm Hg), hot-dry (April, May and mid June, MDT 31.5°C and AVP 11.77 mmHg), hot-humid (mid June to September, MDT 30.1 °C and AVP 22.1 mmHg),comfortable (October to November, MDT 22.5 °C and AVP 14.62 mmHg) and cool (December to January, MDT 13.5 °C and AVP 9.48 mmHg). Data were analyzed statistically according to Snedecor and Cochran (1968).

The average post partum oestrus interval in Indian goats was 205.2 ± 6.6 days (Table 1). Marwari goats had relatively short (182.5± 15.0d) postpartum oestrus interval, followed by 207.1± 7.9 day in Sirohi and longest of 222.1± 15.0 day in Kutchi (Table 1). The difference between breeds was, however, statistically nonsignificant. Sahni and Roy (1967) also indicated that the breeds of smaller size had relatively short postpartum oestrus period. The values observed in Marwari are in close agreement with earlier findings in the same breed (Mittal 1991).

The postpartum oestrus interval was not affected by parity of the animal (Table 2).The mean postpartum interval was almost similar in both primiparous and pluriparous goats.

Season of kidding had significant effect ($P<0.01$) on postpartum oestrus interval in goats. The does kidded during hot-humid season (mid June to September) had shortest postpartum interval of 79.5±14.5 days closely followed by those kidded during hot- dry season (96.8±18.6 days). Does kidded during February to March (comfortable season) had longest interval of 263.0±32.1 days. This trend was suggestive of seasonality in oestrus in these breeds of goat. Mishra and Biswas (1966); Singh *et al.* (1978, 1979) and Singh (1997) have also reported 2 oestrus seasons in these breeds. Similar trend in oestrus occurrence was reported by Singh and Sengar (1979) in Jamunapari, Beetal, Barbari and Black Bengal goats and Rajkonwar and Borgohain (1978) in Assam goats.

Similarly goats kidded during October to January, i.e. comfortable and winter season had relatively longer postpartum oestrus interval compared to those kidded during April to September (summer).

SUMMARY

Postpartum oestrus interval did not differ significantly among breeds. Parity also had no effect. Kutchi had longest (222.1±15.1 day), while Marwari had shortest (182.5±15.0 day) postpartum oestrus interval. Sirohi had intermediate value (207.1±7.9 day). Irrespective of breeds, average postpartum oestrus interval was 205.2 ±6.6 day. Season had significant effect on postpartum oestrus interval. Goats that kidded during October and November had longest (219.3±5.7 day) while goats that kidded during mid June to September had shortest (79.5±14.5 day) postpartum interval.

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Oestrus synchronization in Malabari goats*

M SELVARAJU[1], D KATHIRESAN[2] and T G DEVANATHAN[3]

Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu 600 007 India

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Key words: Goats, Oestrus, Synchronization

The present investigation has been undertaken to study the efficacy of various oestrus synchronizing agents on kidding rate and endocrine pattern in Malabari goats. Malabari does (n=54) were randomly divided into 3 treatment groups viz., FGA, MAP and CIDR groups, each group comprised of 14 does and were treated intravaginally for 18 days with 45 mg Fluorogestone acetate (FGA), 60 mg medroxy progesterone acetate (MAP) vaginal sponges and controlled internal drug release device (CIDR) containing 0.332 g of natural progesterone, respectively, and the remaining 12 does, which exhibited natural oestrus without any treatment were served as control. At the time of pessary withdrawal 600 IU pregnant mare serum gonadotrophin (PMSG) was administered intra muscularly to all the does. After the withdrawal of sponges or CIDR, does of all the 3 treatment groups observed individually every 4 hr for the signs of oestrus using an apronised buck. Seven does each in FGA and MAP groups and 6 does in CIDR group observed in oestrus were bred with intact buck or artificially inseminated with 0.25 ml of frozen-thawed semen containing 150-200 $\times 10^6$ spermatozoa at 48 hr and 60 hr of sponge or CIDR withdrawal. Six does in control group were bred naturally or artificially inseminated, twice at an interval of 12 hr after observing the oestrual signs. Kidding rate was calculated as number of does kidded to the number of does allowed for natural service (NS) or artificial insemination (AI) in all the groups. Blood was collected from the jugular vein of does in a sterilized test tube at the time of sponge or CIDR insertion, withdrawal and at synchronized oestrus for serum progesterone assay.

In this study, 100 per cent oestrus response was observed after FGA or MAP treatment but 2 out of the 14 goats in CIDR group did not show any oestrus response to CIDR treatment. These 2 goats had serum progesterone levels less than 1 ng/ml at the time of CIDR insertion. Chemineau *et al.* (1982) stated that if exogenous progesterone or progestagen treatment is applied at the beginning of the luteal phase it does not seem to prevent ovulation and leads to failure of response to oestrus synchronization treatment. Further, Senn and Richardson (1992) demonstrated that the progesterone or progestagen and PMSG combination was associated with premature luteal failure and suppression of follicular and luteal activity. Probably, the above observations could be the reason for the failure of response in the 2 goats in CIDR group. But none of the FGA and MAP group does had less than 1 ng/ml of serum progesterone at the time of insertion of these sponges and this might be the reason for 100% response in these groups.

Natural services in FGA, MAP, CIDR groups and control group resulted in 71.43, 57.14, 83.33 and 83.33% kidding respectively. The corresponding values were 57.14, 42.86, 83.33 and 50.00% through artificial insemination. The overall kidding percentage observed in FGA, MAP, CIDR and control groups was 64.29, 50.00, 83.33 and 66.67 respectively. In general, N.S. group of all oestrus synchronized and control groups showed higher percentage of kidding rate when compared to AI groups. Ritar and Salamon (1983) attributed the lesser conception rate in goats with AI to the sperm concentration in the semen used for AI and problem with the transport of frozenthawed spermatozoa through the reproductive tract of the goat. Baril *et al.* (1993) opined that the synchronization of oestrum is not precise enough to allow for fertilization to occur in all the goats when AI is performed at a predetermined time after the end of progestagen treatment. In the present study, among all the treated groups, MAP had low percentage of kidding both in NS and AI. In MAP treated does, asynchrony between the time of ovulation and fixed time of breeding might be the possible reason for the reduced kidding rate. Ishwar and Pandey (1992) stated that PMSG administration increases the ovulation rates but it changes the oviduct and uterine environment. This observation might also be a reason for the lower kidding rate in MAP group in this study.

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Present address: [1]Assistant Professor, Department of Obstetrics and Gynaecology, Veterinary College and Research Institute, Namakkal. 637 001. [2]Professor and Head, Veterinary University Training and Research Centre, Madurai 625 020. [3]Professor, Department of Obstetrics and Gynaecology, Madras Veterinary College, Chennai 600 007

Mean serum progesterone concentrations of 2.42 ± 0.23, 2.64 ± 0.36 and 3.54 ± 0.24 ng/ml were observed at the time of insertion of FGA, MAP sponges and CIDR device respectively. At the time of withdrawal, the mean progesterone concentrations observed in FGA, MAP and CIDR groups were 2.64 ± 0.18, 2.88 ± 0.36 and 3.81 ± 0.26 ng/ml respectively. The overall mean progesterone concentrations observed at the time of insertion of sponges and CIDR device and their withdrawal were 2.87 ± 0.17 and 3.11 ± 0.17 ng/ml respectively. In the present study, the progesterone levels observed at the time of withdrawal of sponges or CIDR were slightly higher than the level observed at the time of sponge or CIDR insertion. Hence, the level of progesterone was maintained in all the treated groups until cessation of treatment, which in turn prolonged the progestational phase in all the treated goats. Similar trend in progesterone level was observed in goats by Chemineau *et al.* (1982). In the present study, the mean progesterone level at standing oestrum was less than 0.51 ng/ml in all the treatment groups. Similar observations were made by Alacam *et al.* (1985) and Pathiraja *et al.* (1991) in oestrus synchronized goats. No difference in the progesterone level at standing oestrum between treatment groups and control group indicated complete luteolysis in all the treated goats. This explains the comparable fertility between oestrus synchronized and control groups.

SUMMARY

Treatment with FGA, MAP and CIDR intra vaginal pessaries resulted in better synchrony of oestrus and improved kidding rate in Malabari goats. Hence, it is concluded that all the 3 treatments can be used for oestrus synchronization in Malabari goats under field conditions.

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Foetal haemoglobin in adult blood of yak (*Bos grunniens*)

N C NATH[1], M BHATTACHARYA[2] and A AZIZ[3]

Assam Agricultural University, Guwahati, Assam 781 022 India

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Key words: Foetal haemoglobin, Yak

The yak is the largest bovid to inhabit at high altitudes between 3000 to 5000 m above mean sea level where oxygen tension is very low. Sarkar *et al.* (1999) reported that the survival of the yak at low oxygen tension areas could be due to presence of foetal haemoglobin in adult yak, which they studied by chemical (alkali resistant) method. In the present study, an attempt was made to confirm the presence of foetal haemoglobin (Hb F) in adult yak by chemical method and electrophoretic behaviour of haemoglobin.

Blood samples were collected by jugular venipuncture into EDTA tube (1 mg/ml of blood) from 5 adult yaks 2 – 3 years of age in the Department of Anatomy, College of Veterinary Science, Guwahati, Assam. Total haemoglobin was estimated by acid hematin method (Jain 1986). The presence of foetal haemoglobin was screened by resistance to alkali denaturation, which is the most widely used method (Varley 1976) to determine the foetal haemoglobin content in adult blood. From freshly collected blood, plasma protein free haemolysate at the concentration of 10 g/dl was prepared and presence of Hb F was screened (Varley 1976). Polyacrylamide gel electrophoresis of haemolysate at alkaline buffer (tris glycine, 0.03 M, pH 8.6) in non-dissociating system was done (Davis 1964). To visualize individual globin chain of haemoglobin, electrophoresis of urea treated haemolysate was done in the above gel and buffer containing 6 M urea (dissociating system). This is the excellent method to visualize individual globin chain (Carrell and Lehman 1990).

The estimated total haemoglobin level was 12 ± 0.45 g%. The percentage of Hb F was 38.29 ± 0.84. Polyacrylamide gel electrophoresis in non-dissociating system revealed 2 red bands migrating in the gel which after staining with amido black indicated the presence of only those 2 bands on the gel (Fig. 1A). This distinctly indicated that the haemolysates were free from any contaminated protein. The bands were regarded as HbA and HbB. The electrophoretic pattern in dissociating system revealed 4 major bands (Fig. 1B) with relative

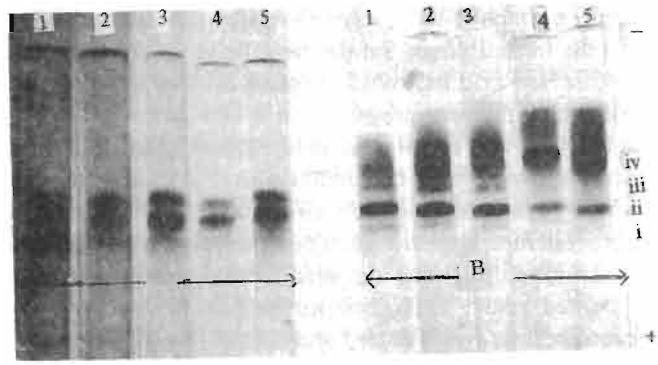

Fig. 1. Polyacrylamide gel (7%) electrophoresis of blood haemolysate of 5 adult yaks. (A) non-dissociating system; (B) dissociating system.

electrophoretic mobilities of 0.59, 0.56, 0.49 and 0.43, and were regarded as i, ii, iii and iv. Two minor protein bands with relative electrophoretic mobilities of 0.84 and 0.21 were also seen.

Presence of Hb F in adult yak blood (42.9% of total Hb) was reported by Sarkar *et al.* (1999) by chemical method. Adult blood of white tailed deer also contains Hb F (Jain 1993). Generally, bovine haemoglobin is of 2 types – highest mobility type is HbA followed by type HbB (Winter *et al.* 1984). Presence of 4 major polypeptides in dissociating system indicated that adult yak blood contains Hb F. In bovine, concentration of Hb F ($\alpha_2\nu_2$) is the highest at birth and generally diminishes with age as it is replaced by adult haemoglobin ($\alpha_2\beta_2$) within a few weeks of life in most animals and in a few months in some. Along with major adult haemoglobin ($\alpha_2\beta_2$), a minor haemoglobin type ($\alpha_2\sigma_2$) is also present in adult blood (Jain 1993). Therefore separation of 2 bands on electrophoresed gel using dissociating agent urea in the present study is indicative of presence of α and ß globin chains, whereas three bands indicated the presence of α, ß and ν or α, ß and σ. Similarly, presence of 4 bands indicated the presence of α, ß, ν and σ, i.e. presence of Hb F ($\alpha_2\nu_2$) along with normal adult haemoglobin.

The presence of Hb F in adult blood of yak can be attributed

Present address: [1]Department of Physiology and Biochemistry, [2]Department of Animal Genetic and Breeding, College of Veterinary Science. [3]Director, NRC on Yak, Dirang, Arunachal Pradesh

as high altitude adoptive mechanism. Hypoxic condition at high altitude results in a marked increase in erythropoetic production and release which in turn enables blood cell production and synthesis of foetal haemoglobin (Jain 1993). Foetal haemoglobin both takes up oxygen and loses carbon-di-oxide more easily at low oxygen tension than does the adult form (Varley 1976).

SUMMARY

Blood samples from 5 adult yaks (2 – 3 years of age) were collected to screen the presence of Hb F by chemical method, as well as by polyacrylamide gel electrophoresis. The total haemoglobin content in adult yak blood was 12.40 ± 0.45 g%. Presence of alkali resistant Hb in the adult yak blood and presence of 4 globin chains in haemolysate observed in the electrophoresed gel indicated that adult yak blood contained Hb F. The percentage of Hb F of total Hb was 38.29 ± 0.84.

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Hypophyseal biometry and major adenohypophyseal proteins of yak (*Bos grunniens*)

N C NATH[1] and M BHATTACHARYA[2]

Assam Agricultural University, Guwahati, Assam 781 022 India

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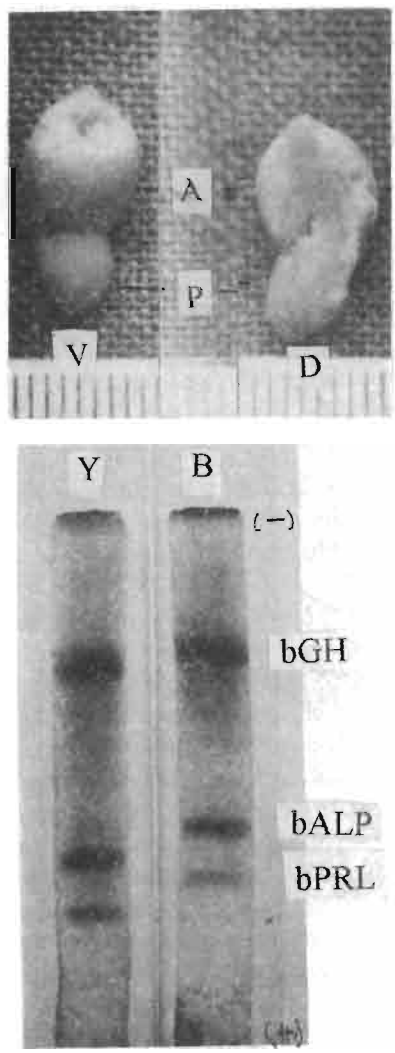
Key words: Hypophysis, Biometry, Adenohypophyseal protein, Yak

Biometry of pituitary and polyacrylamide gel (7%) electrophoretic pattern of anterior pituitary proteins of yak, a species about which there is little information, are presented in this paper.

Pituitaries from 3 adult bull yak (2-2.5 years-old, 80-95 kg body weight) were collected immediately after sacrifice kept for anatomical studies in the Department of Anatomy, College of Veterinary Science, Guwahati, Assam. The pituitary of the yak (Fig. 1) was irregularly conical in outline. On gross examination, the pituitary consisted of 2 parts, which can be distinguished as anterior pituitary and posterior pituitary by colour. The oval shaped, reddish brown colour anterior pituitary formed the major portion of the gland, comprised on an average 67% (0.14 g) of the total pituitary weight (0.21g) and comprised 67% (2 cmm) of the total pituitary volume (3 cmm). The rest 33% (0.07 g) weight and 33% (1 cmm) volume comprised by posterior pituitary and infundibulum stalk. The maximum length, width and thickness (including infundibulum stalk) of anterior pituitary were 93.33 ± 1.66 mm, 70.67 ± 1.19 mm and 36.00 ± 0.47 mm respectively. The corresponding measurements for posterior pituitary were 56.67 ± 1.66 mm, 41.00 ± 1.70 mm and 28.33 ± 1.19 mm. The infundibulum spanned the entire dorsal surface of the anterior pituitary. The conical shaped, pale colour posterior pituitary was not surrounded by anterior pituitary and it was projecting out from the distal end of the anterior pituitary, as a result, the total length (1.49 cm) of the pituitary was formed by the combination of the anterior and the posterior pituitary. The gross anatomy of the pituitary of yak was comparable to that of sheep (Venzke 1977).

Alkaline extract (0.1 M ammonium bicarbonate, pH 8.6) of fresh anterior pituitary tissue of yak and cow (bovine) were prepared (Nath and Singh 1992). Polyacrylamide gel (7%) electrophoresis of pituitary proteins was performed in tube (10 cm × 0.5 cm) using tris (hydroxymethyl) methylamine



Figs 1-2. **1.** Pituitary of an adult yak. V, veratral view: D, dorsal veiw, A, anterior pituitary, P, posterior pituitary. **2.** Polyacrylamide gel (7%) electrophoretic (pH 8.6) pattern of alkaline extract (pH 8.6) of anterior pituitary tissue proteins (100µg) of yak. B, Bovine. Y, yak; bGH, bovine growth hormone, bACP, bovine albumin live protein; bPRL, bovine prolactin.

Present address: [1]Professor, Department of Animal Physiology and Biochemistry.

[2]Director, NRC on Yak, Dirang, Arunachal Pradesh.

glycine buffer (0.04 M, pH 8.6) as described by Davis (1964). Pituitary proteins (200 µg) was applied for electrophoresis. Electrophoresis was performed at a constant current 1 mA/gel tube for 15 min and then increased to 3 mA/gel tube (200-250 V) for 2 hr till tracking dye (bromophenol blue, 0.05%) reached the lower end of the gel. The gels were stained with 1% amido black in 7% acetic acid. Destaining was done by several changes of 7% acetic acid.

The electrophoretic pattern of major anterior pituitary protein of bovine has been studied previously (Nath and Singh 1992) using standard bovine growth hormone (bGH) and bovine prolactin (bPRL) from National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, Baltimore, Maryland, USA. The protein bands of yak (Fig. 2) were identified by comparing with bovine. In bovine, the relative electrophoretic mobilities for GH, ALP (albumin-like protein) and PRL were 0.26, 0.59 and 0.68 respectively. Three major protein bands corresponding to bHG, bALP and bPRL were seen in yak with relative electrophoretic mobilities 0.29, 0.52 and 0.64 respectively. The band having electrophoretic mobility intermediate to GH and PRL was named as albumin (Sinha and Baxter 1979, Talamantes *et al.* 1981) or albumin-like protein (Nath and Singh 1992) in many other species of animals due to the same molecular weight as serum albumin (66 kd). One or two minor protein bands just ahead of GH and PRL were also seen. One or two minor protein bands moving just ahead of GH and PRL were also observed in standard bGH and bPRL (Nath and Singh 1992) and in freshly prepared anterior pituitary alkaline tissue homogenate of many species of animals (Russel *et al.* 1978, Nath and Singh 1992). Russel *et al.* (1978) and Talamantes *et al.* (1981) claimed that these doublets were probably deaminated forms of GH and PR. The difference in electrophoretic mobilities of bGH, bALP and bPRL with that of yak GH, ALP and PRL can be attributed to many factors. The electrophoretic mobility of protein at alkaline pH was inversely related to pI value of protein. Molecular changes such as deamination or substitution of charged for an uncharged amino acid side chain would also induce such changes in pI and electrophoretic mobility (Wallis 1973).

SUMMARY

Biometry of hypophysis and polyacrylamide gel (7%) electrophoretic (pH 8.6) pattern of alkaline extract (pH 8.6) of adenohypophyseal proteins of adult yak (*Bos grunniens*) was studied. The gland was irregularly conical in outline and comprised anterior and posterior parts. The anterior pituitary constituted 67% of total weight (0.21 g) as well as total volume (3 cmm) of the gland, the rest (33%) was posterior pituitary and infundibulum stalk. Polyacrylamide gel (7%) electrophoretic (pH 8.6) pattern of alkaline extract (pH 8.6) of anterior pituitary tissue protein revealed 3 major protein bands, viz. growth hormone, albumin-like protein and prolactin with relative electrophoretic mobilities of 0.29, 0.52 and 0.64, respectively, which were different from bovine growth hormone (0.27), bovine albumin-like protein (0.59) and bovine prolactin (0.68).

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Molecular characterization of indigenous cattle breeds by randomly amplified polymorphic DNA fingerprinting

T K BHATTACHARYA[1], PUSHPENDRA KUMAR[2] and J D JOSHI[3]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

The study was carried out in Tharparkar, Hariana and Red Sindhi cattle maintained at several herds in India. The animals were characterized using randomly amplified polymorphic DNA fingerprinting using short oligonucleotide primers. All the primers showed polymorphic pattern under study. The intrabreed similarity was highest (0.86 ± 0.04) in Red Sindhi population followed by Tharparkar (0.76 ± 0.02) and Hariana (0.75 ± 0.04). But, interbreed similarity was highest between Tharparkar and Hariana (0.68 ± 0.04) followed by Red Sindhi-Tharparkar (0.66 ± 0.03) and Hariana-Red Sindhi (0.62 ± 0.04). Another approach for identifying breed differences are average percentage difference which was highest between Red Sindhi-Hariana (37.39 ± 3.31) followed by Red Sindhi-Tharparkar (34.11 ± 3.35) and Hariana-Tharparkar (33.31 ± 3.38). Thus, RAPD not only explains intrabreed variation but interbreed difference too.

Key words: Cattle, Characterization, DNA fingerprinting, Hariana, RAPD, Red Sindhi, Tharparkar

The most important local breeds in India are Hariana, Tharparkar and Red Sindhi. They are having potentiality for high milk yield and draught. They are hardy, tolerant to tropical heat and harsh environment, resistant to diseases particularly parasitic diseases, surviving by taking poor quality nutrition as well as scanty drinking water and yielding a good quality beef. But, to meet the demand of milk, crossbreeding was followed since 1929 in the country. Due to such practices, these indigenous stocks were decreasing gradually and they become threatened. Hence, it is important to quantify the magnitude of genetic differences between them. Indication of their genetic differences will allow to formulate proper breeding programme to enhance the productivity of milk and will also be of value in conservation of these breeds. To characterize these breeds we should have adequate knowledge about genetic variation present within and between population. However, to estimate such variability a quick, reliable and economically viable technique is random amplified polymorphic DNA fingerprinting (RAPD), which implies amplification of DNA by using random primers (Welsh and McClelland 1990, Williams *et al.* 1990, Kantanen *et al.* 1995). This was extensively used in honey bee (Hunt and Page 1992), insects (Landry *et al.* 1993) and for constructing phylogenetic tree, genetic mapping (Cockett and Medrano 1996) and linkage analysis (Martin *et al.* 1991). Apart from this, RAPD was also applied to identify marker for QTL (Michelmore *et al.* 1991), species (Koh *et al.* 1998), breed (Yang *et al.* 1996, Carpio *et al.* 1996) and sex (Antoniou *et al.* 1994, Antoniou and Skidmore 1995, Gutierrez *et al.* 1997). The major advantage of this technique is identification of informative markers without prior knowledge of sequence information. Thus, the aim of this study was to estimate RAPD variation in Indian cattle breeds which could be of use in characterizing them.

Present address: [1]Scientist, [2]Senior Scientist, [3]Formerly Principal Scientist, Animal Genetics Division.

MATERIALS AND METHODS

Animals

The study was carried out on representative animals of 3 cattle breeds in India (Hariana, Tharparkar and Red Sindhi). The samples were collected randomly from 20 animals of both sexes of each breed from several herds.

DNA samples

Blood samples were collected in 50 ml sterile polypropylene vial containing 0.5ml of 0.5 M EDTA and DNA was isolated as per Bhattacharya (1999). Quality of DNA was checked by running DNA in 1% w/v low EEO agarose with ethidium bromide staining (0.5 µg/ml), whereas quantity was examined with spectophotometry by taking reading at OD_{260}. Samples were adjusted to a concentration of 20 µg/ml before commencing PCR reactions.

DNA amplification

Genomic DNA was amplified in PTC-200 programmable

thermal cycler using publicly available 9 oligonucleotide primers (Table 1). Each 50 µl PCR reaction mix comprised 60-90 ng of DNA; 100µM dNTPs; 40 ng primer; 1 U Taq polymerase enzyme; 2 mM $MgCl_2$; 100 mM tris-HCl, pH 8.8; 500 mM KCl. Following an initial denaturation step at 94°C for 3 min, the reaction was subjected to 40 cycles denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 1 min followed by final extension at 72°C for 5 min. PCR products were electrophoresed at 150 volt in 1.6% w/v low EEO agarose containing ethidium bromide with 1X tris-boric acid-EDTA buffer. RAPD fingerprints were visualized under UV-transilluminator and documented by photography. Only distinct and prominent bands were scored from the gel. RAPD fragments were compared only on samples run in the same gel.

Table 1. RAPD primers producing polymorphic bands

Primer	Sequence (5' to 3')	Length	GC%
A1	GCACTGAGTA	10	50
A2	ACGTCGAGCA	10	60
A5	GAATCGGCCA	10	60
P1	ACAACGCCTC	10	60
P2	GGGAACGTGT	10	60
P3	CTGGGCAACT	10	60
P4	CCGTGACTCA	10	60
T3	GCTGCTCGAGT	11	64
T4	AACCGCGGTCT	11	64

Data analysis

The RAPD bands were used to estimate band sharing (BS), percentage difference (PD), average percentage difference (APD) and mean average percentage difference (MAPD). Band sharing or similarity coefficients within and between breeds were calculated as an expression of similarity of RAPD fingerprints of animals from the same or different breeds (Wetton *et al.* 1987, Jeffery and Morton 1987, Dunnington *et al.* 1990). Mathematically it could be expressed as BS=2Cab/(Na+Nb), where, Cab is the number of common bands showed by individual a and b; Na and Nb are the total number of bands for individual a and b respectively. Mean average percentage difference (MAPD) was estimated as differences among breeds using 3 formula (Gilbert *et al.* 1990), PD=Dab/(Na+Nb) x100; APD=1/PÓ Pdi; MAPD=1/RÓ APDj, where, Dab is the number of fragments differed between 2 individuals a and b for each primer; Na and Nb are the total number of bands in individual a and b; P is the inter-breed pair comparisons; R is the number of random primers used.

RESULTS AND DISCUSSION

RAPD fingerprints

All the 9 primers exhibited satisfactory amplification and polymorphic bands whose size was ranging from 0.15 to 3.00 kb. The number of resolvable bands across the primers were counted in range of 2 to 18 (Table 2). The A1 primer produced bands varied from 9 to 14 in Hariana, 7 to 14 in Tharparkar and 7 to 13 in Red Sindhi cattle. With this primer most of the bands were found as monomorphic, whereas only 2 bands were polymorphic being size of 0.25 to 2.02 kb. In A2 primer 7 bands were polymorphic, whereas 2 polymorphic bands were observed in T3 primer. The T4 primer showed 7 bands to be polymorphic whose size varied from 0.15 to 3.00 kb. The other primers, A5, P1, P2, P3 and P4 revealed 7, 5, 4, 4 and 2 polymorphic bands.

Table 2. Average number of bands per primer in different breeds (n=20)

Primer	Hariana (mean ± SE)	Tharparkar (mean ± SE)	Red Sindhi (mean ± SE)	Overall mean
A1	11.89±0.31 (9-14)	10.39±0.51 (7-14)	11.06±0.37 (7-13)	11.11
A2	8.36±0.59 (6-12)	8.56±0.34 (5-12)	8.22±0.56 (4-14)	8.38
A5	9.94±1.21 (3-18)	12.83±0.80 (6-18)	12.72±0.74 (7-18)	11.83
T3	9.06±0.49 (4-12)	9.89±0.62 (6-13)	7.56±0.54 (4-11)	8.84
T4	12.11±0.62 (7-15)	8.89±0.72 (3-14)	6.17±0.29 (4-8)	9 06
P1	9.61±0.75 (4-14)	9.67±0.53 (5-14)	8.41±0.84 (3-14)	9.23
P2	6.94±0.57 (3-10)	7.11±0.77 (1-12)	6.17±0.70 (1-10)	6.74
P3	6.94±0.77 (2-12)	8.17±0.42 (4-11)	6.33±0.51 (2-9)	8.20
P4	7.83±0.09 (5-10)	8.06±0.34 (5-10)	8.72±0.49 (4-12)	
Overall	9.19±0.64	9.219±0.56	8.37±0.75	8.95

Values within parenthesis are the range of appearing bands in the individuals within a breed using individual primer, n indicate the number of animals under each breed.

Within breed similarity

It was calculated on RAPD fingerprints generated from individual DNA analysis (190 pairs / primer). The average similarity or band sharing was 0.86 ± 0.04 in Red Sindhi, 0.76 ± 0.02 in Tharparkar and 0.75 ± 0.04 in Hariana cattle (Table 3) depicting the greater homogeneity within the population. If we see primer-wise band sharing, the highest magnitude was with A1 primer and lowest one from A2 primer. However, overall 60% or more band sharing was found across the primer. These all were the indicative of genetic purity within the population.

Between breed similarity

Inter-breed similarity index or band sharing was highest between Hariana and Tharparkar followed by Red Sindhi-

Table 3. Average band sharing frequencies (R) in 3 Indian zebu cattle population

Primer	Hariana	Tharparkar	Red Sindhi
A1	0.95 ± 0.05	0.83 ± 0.03	0.90 ± 0.01
A2	0.74 ± 0.03	0.76 ± 0.02	0.68 ± 0.03
A5	0.75 ± 0.02	0.77 ± 0.02	0.86 ± 0.01
T3	0.79 ± 0.02	0.73 ± 0.04	0.76 ± 0.03
T4	0.73 ± 0.03	0.69 ± 0.03	0.79 ± 0.02
P1	0.75 ± 0.02	0.73 ± 0.02	1.00 ± 0.06
P2	0.60 ± 0.03	0.66 ± 0.04	1.00 ± 0.04
P3	0.62 ± 0.04	0.80 ± 0.02	0.86 ± 0.03
P4	0.83 ± 0.02	0.84 ± 0.02	0.86 ± 0.02
Overall	0.75 ± 0.04	0.76 ± 0.02	0.86 ± 0.04

Tharparkar and Hariana-Red Sindhi (Table 4). These indices indicated the genetic relatedness among breeds/populations. Out of the 9 primers, A1, A2, T3, P1 and P4 exhibited a greater amount of band sharing in Hariana-Tharparkar pair whereas T3, T4, A5, P1, P3 and P4 depicted maximum resemblance in Red Sindhi-Tharparkar pair. On the otherhand, we observed maximum genetic dissimilarities (MAPD) between Hariana and Red Sindhi cattle (37.39 ± 3.31) and lowest magnitude was found between Hariana and Tharparkar population (Table 5). Kantanen *et al.* (1995) made an attempt to estimate genetic variability in cattle in Finland. Within breed variability (intra-breed similarity indices) was highest in Red Sindhi population which indicated homogeneity within the population.

Table 4. Average band sharing frequencies between breeds

Primer	Hariana-Tharparkar (mean ± SE)	Hariana-Red Sindhi (mean ± SE)	Red Sindhi-Tharparkar (mean ± SE)
A1	0.90 ± 0.02	0.85 ± 0.01	0.81 ± 0.02
A2	0.64 ± 0.02	0.55 ± 0.01	0.55 ± 0.01
A5	0.62 ± 0.02	0.60 ± 0.01	0.75 ± 0.01
T3	0.62 ± 0.02	0.55 ± 0.02	0.58 ± 0.02
T4	0.60 ± 0.02	0.51 ± 0.01	0.61 ± 0.01
P1	0.74 ± 0.01	0.65 ± 0.02	0.67 ± 0.02
P2	0.55 ± 0.02	0.56 ± 0.02	0.56 ± 0.02
P3	0.63 ± 0.02	0.59 ± 0.02	0.71 ± 0.02
P4	0.82 ± 0.01	0.71 ± 0.01	0.73 ± 0.01
Overall	0.68 ± 0.04	0.62 ± 0.04	0.66 ± 0.03

Inter-breed similarity indices were taken into account to find out genetic similarity between breeds. The highest similarity was found between Hariana and Tharparkar breeds. Phenotypically these 2 breeds are having similarity in terms of physical stature, yielding potentiality, workability, behaviour-wise. Similarly, mean average percentage difference indicate the breed differences or in the other words the dissimilarity between breeds being an indirect measurement of similarity indices. These results got some similarity to the findings in zebu cattle of Finland (Kantanen *et al.* 1995). As the inter-breed similarity indices were high among these Indian breeds, it might be possible that these breeds were evolved recently. As time will go, the segregation and mutation would get upper hand to create more distance among each other. It is relatively easy and quick to segregate and fix major genes responsible for qualitative characters which may be the distinguishable features of the breeds. But, for minor genes it took thousands and thousands years to fix a character properly to show breed/species specifically.

Table 5. Average percentage difference (APD) and mean average percentage difference (MAPD) between breeds

Primer	Hariana-Tharparkar (mean ± SE)	Hariana-Red Sindhi (mean ± SE)	Red Sindhi-Tharparkar (mean ± SE)
A1	18.97 ± 1.55	15.55 ± 1.15	19.29 ± 1.73
A2	34.63 ± 1.70	43.47 ± 1.45	42.79 ± 1.37
A5	38.36 ± 1.86	39.20 ± 1.30	26.70 ± 1.75
T3	37.36 ± 1.86	39.20 ± 1.30	26.70 ± 1.75
T4	40.29 ± 1.50	47.99 ± 0.99	39.39 ± 1.12
P1	25.73 ± 1.34	35.36 ± 1.83	32.72 ± 1.78
P2	48.55 ± 2.14	42.67 ± 1.97	51.14 ± 2.69
P3	37.33 ± 1.95	39.20 ± 1.89	28.77 ± 1.75
P4	18.56 ± 1.33	28.63 ± 1.42	25.68 ± 0.98
MAPD	33.31 ± 3.38	37.39 ± 3.31	34.11 ± 3.35

The similarity index might underestimate the population heterozygosity in particular when alleles are novel (Lynch 1991). The exact magnitude of the biasness can not be determined due to dominant nature of alleles. Thus, allele frequency too can not be estimated directly. However, this study authenticate the breeds in terms of RAPD-fingerprints.

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Factors affecting first lactation traits, expected breeding value and breeding efficiency in Karan Fries cattle

M K SINGH[1] and M GURNANI[2]

National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

The present study was carried out in Karan Fries strain of cattle maintained at the NDRI, Karnal. Data were classified on the basis of sire, level of inbreeding, season and period. The effect of sire was significant on FLY, TFLY, MY/FLL, MY/FCI and EBV ($P<0.01$) and 10 and 15% sires had superiority over herd average of 11 and 7% for FLY and 25 and 15% for TLFY. The effect of level of inbreeding was significant on FLY, TFLY, MY/FLL and MY/FCI ($P<0.05$). Decrease in production performance of above these traits was recorded with the increase in level of inbreeding, though the effect was significantly adverse in highly inbred ($F>12\%$) group of animals. Deterioration in reproductive performance was also recorded in highly inbred cows. Inbreeding had more effect on first lactation performance than later lactations. The regression analysis, however, showed adverse but nonsignificant effect of inbreeding in most of the production traits and significant on FSP, FCI and MY/FCI ($P<0.05$). The effect of season of calving was significant on FLL, FSP and FCI ($P<0.05$). Summer calvers had maximum lactation length, whereas, autumn calvers had minimum FSP and FCI. The FSP, FCI, MY/FLL and MY/FCI were significantly influenced by period of calving. Significantly consistent increase in milk production efficiency traits was observed over the periods and availability of daughters of some progeny tested sires in last period might be the reason of it. The overall findings revealed large variation in transmitting ability of sires for production traits, adverse effect of high level of inbreeding on performance and fluctuation in performance due to both genetic and non-genetic factors, therefore study suggests use of progeny tested sires, keeping of inbreeding under safer limit and management intervention for consistent improvement of herd.

Key words: Cattle, Inbreeding, Performance, Regression, Sire

Crossbreeding with elite sires in many parts of the country made dairy farming commercially viable. It is therefore necessary to have a nucleus herd of high genetic merit to meet the requirement of crossbred bulls under field conditions. Such a nucleus herd may follow closed breeding like Karan Fries herd. In a closed breeding system after 6-7 generations with limited number of sires, there is likelihood of increase in inbreeding and subsequent ill effect on performance of the animals. This herd has shown fluctuation in terms of production and reproduction during last few years. Inbreeding with certain other factors has been named for this situation. Therefore, it would be useful to investigate the knowledge of production level and effect of inbreeding, and other genetic and non-genetic factors on production and reproduction records, so that appropriate breeding programmes can be made for bringing about genetic improvement in the herd. Hence the present investigation was taken to study effects of genetic sire, level of inbreeding) and non-genetic factors (season and period of calving) on performance traits.

Present address: [1]Scientist, CIRG, Makhdoom, Farah, Mathura 281 122.

[2]Formerly Principal Scientist and Head, Dairy Cattle Breeding, 246, Old Housing Board, Sector-13, Karnal.

MATERIALS AND METHODS

The study was carried out on the Karan Fries strain of cattle developed at the National Dairy Research Institute, Karnal. The semen of progeny tested Holstein Friesian bulls were used to cross Tharparkar cows; crossbreeding followed by inter-se mating to develop the strain. Data were collected from 490 cows, progeny of 32 bulls born during 1980 to 1990 and calved 1982 to 1992. Pedigree chart was prepared for every animal and the pedigree of animals was traced back up to foundation stock assuming their inbreeding coefficient as zero. The coefficient of inbreeding for every animal was estimated by path coefficient method (Wright 1922). Data were classified into 5 periods of 2 years each except the VI period (3 years due to comparatively small number of observations) to minimize the management and climatic fluctuations. The year was sub divided into 5 seasons i.e winter (Dec- Jan), spring (Feb-March), summer (Apr-June),

rainy (July-Sep) and autumn (Oct-Nov) based on relative humidity and ambient temperature. The animals were grouped into 4 levels of inbreeding, based on their inbreeding coefficient i.e. non-inbred (Fx: 0), lowly inbred (Fx; ≤6%), marginally inbred (Fx; >6≤12%) and highly inbred (Fx; >12%). The effect of various factors on the first lactation performance traits was investigated by the least- squares analysis of variance (Harvey 1975) by a model which included sire, inbreeding level, season and period of calving as fixed effects and age at first calving (AFC) as co variable as explained below.

$X_{ijklm} = \mu + Ii + Sj + P_k + Sl + b_{xA}(Aijklm-\bar{A}) + eijklm$

Where,

X_{ijklm} is performance of mth cow, ith inbreeding group, jth sire, kth period of calving and lth season of calving, μ is population mean, Ii is effect of level of inbreeding, Sj is effect of sire, Pk is period of calving, Sl is season of calving, b_{xA} regression of Xijklm on AFC (Aijklm), $\bar{A}$ is average age of first calving, and eijklm is residual random error with 0 mean and variance δ^2 (NID) $(0,\delta^2)$. However, for estimating EBV and EBE the AFC was not included in the model and pooled data for all available records (minimum 2 complete records) were used for analysis. Breeding efficiency was estimated as per Wilcox *et al.* (1957). The differences among sub class means were tested by DMRT (Kramer 1957). The heritability (h^2) was estimated on adjusted data by paternal halfsib correlation method (Becker 1985). The abnormal and incomplete records resulted from chronic or incurable diseases were excluded from this study. The sires having a minimum of 5 progeny were included for this study. The traits investigated were first lactation yield 305 days or less (FLY), total first lactation yield (TFLY), first lactation length (FLL), first service period (FSP), first dry period (FDP), first calving interval (FCI), milk yield per day of first lactation length (MY/FLL), milk yield per day of first calving interval (MY/FCI), expected breeding value (EBV) and expected breeding efficiency (EBE).

RESULTS AND DISCUSSION

The least- squares analysis of variance with respect to different sources of variation and least squares means are presented in Table 1, 2. The estimates obtained for production performance traits were higher than estimates reported by Jadhav *et al.* (1991), Raheja (1994) and Singh *et al.* (2000). However, Dhangar and Patel (1991) obtained little higher breeding efficiency in Jersey crosses. The coefficient of variation in TFLY was 7.0% higher than that of FLY. This is because TFLY is based on actual lactation length which is 8.5% higher than standard lactation length of 305 days, because many cows did not settle in pregnancy in time after calving and so the effect of management factors are expected to be higher and hence, there is more variation in TFLY.

Sire effect

The least-squares analysis of variance revealed significant effect of sire on FLY, TFLY, MY/FLL, MY/FCI and EBV ($P<0.01$, Table1). The sires showing 10 and 15% superiority over herd average were 11, and 7 for FLY, 25 and 15 for TFLY, 10 and 4 for MY/FLL, 18 and 7 for MY/FCI and 11, 7% for EBV respectively. The rank correlation of FLY with TFLY and EBV for sires was 0.89 indicating that ranking of sire on the basis of either trait was equally effective. The results indicated that selection on the basis of FLY would be about 80% as accurate as that on the basis of either TFLY or EBV. The rank correlation between MY/FLL and MY/FCI for sires was 0.78 indicating that ranking of sires on average MY/FLL were about 64% as accurate as that based on MY/FCI. Garcha *et al.* (1991) and Raheja (1994) also observed significant effect of sire on milk production traits in different breeds of cattle. The effect of sire was nonsignificant on FLL, FSP, FDP, FCI and EBE, which indicated that these traits were more under the influence of management and other environmental factors rather than genetic factors.

Level of inbreeding

The average inbreeding coefficient of inbred females. average annual increase and their inbreeding coefficient were 6.65, 1.10 and 0.37% respectively. The level of inbreeding had significant effect on FLY, TFLY, MY/FLL and MY/FCI ($P<0.05$, Table1) and nonsignificant on EBV, indicating that inbreeding had greater affects on first lactation traits than later lactations. A consistent decreasing trend observed on the performance of these traits with the increase in level of inbreeding. However, no significant difference was obtained in milk performance among non-inbred, low and marginally inbred. The highly inbred cows (F; >12%) showed 16 and 12% lower performance than non-inbred for FLY and TFLY respectively. The regression of FLY, TFLY, MY/FLL and MY/FCI on inbreeding coefficient was also negative but non-significant except MY/FCI ($P<0.05$, Table 3), with high magnitude of estimate i.e. 0.5 kg milk reduced by 1% increase in level of inbreeding. Miglior *et al.* (1992) and Thompson *et al.* (2000) in exotic cattle, and Malik *et al.* (1967), Srinivas and Gurnani (1981) and Singh and Nagarcenkar (1997) in zebu cattle have also reported significantly adverse effect of high level of inbreeding on milk production traits. The least-squares analysis illustrated non-significant effect of inbreeding on FLL, FSP, FDP, FCI and EBV; however, inbreeding above 12% had non-favourable effect on the performance of these traits. The results were in agreement with the findings of Gurnani *et al.* (1971), Khanna *et al.* (1979) and Reddy and Sampath (1989) in Haryana, Sahiwal and Red Sindhi cows. Regression analysis also showed declining but nonsignificant effect of inbreeding in above traits except FSP and FCI, where its magnitude was small ($P<0.05$, Table 3). The result was in conformity with those of Reddy and Nagarcenkar (1988) and Kaygisiz (1997) on FLL, FSP, FDP, and FCI. Extensive use of some sires and the close relationship among sires in this herd were mainly accounted for built-up of inbreeding. It

might also had offset genetic gains due to losses in survivability, growth and hence production. Nevertheless, it may also affect the accuracy of breeding value of sires for the selection. The results indicated that higher level of inbreeding had the depressing effect on performance traits in this herd. These results suggested that bulls were not used for very long duration and high level of inbreeding should be avoided unless the genetic potential of mating is more.

Season of calving

The season of calving had significant influence on FLL, FSP and FCI ($P<0.05$) and, its effect was nonsignificant on other performance traits (Table1). Maximum lactation length was observed for summer calvers followed by spring and autumn calvers and the lowest for winter calvers, which was also significantly different from other season calvers (Table 2). Longer lactation period of spring and summer calvers could be due to higher FSP of such cows. The lowest FSP and FCI were observed for autumn calvers followed by winter calvers. The averages of FSP and FCI for summer calvers were highest and also significantly differed from other season calvers. The longer service period for summer calvers could be because of high environmental stress. The summer calvers had longest calving period, which might be due to longest service period of such cows. High genetic correlation of FSP with FCI (0.89± 0.05) in this herd had further support this finding. Significant effect of season on FLL, FSP and FCI was also reported by Jadhav *et al.* (1991).

Period of calving

The effect of period of calving was significant on FSP, FCI, MY/FLL and MY/FCI (Table 1). Least- squares means across the periods revealed significantly lower FSP and FCI for period 2 as compared to those of periods of 3 and 4, and no consistent trend for both traits was observed over the periods. This fluctuation in FSP could be due to changes in environmental conditions over period of calving. The variability in FCI over periods might be due to it's high association with FSP, since similar trend was observed for service period. These findings were in agreement with Chaudhary *et al.* (1995). A consistent increasing trend for MY/FLL and MY/FCI were recorded over the periods, which could be because of use of high breeding value progeny tested sires and better management practices. Garcha *et al.* (1991), Arora *et al.* (1993) and Singh *et al.* (2000) also observed significant effect of period of calving on these efficiency traits. The effect of period of calving was nonsignificant on other first lactation traits, EBV and EBE.

Table 1. Least-squares analysis of variance of performance traits in Karan Fries cattle

Traits	Source of variation											
	Sire		Inbreeding		Season		Period		Regression		Error	
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
FLY (kg)	31	1005439.1**	3	2017328.0*	4	125822.5	4	733461.4	1	1958204.3	449	533413.6
TFLY (kg)	31	2231874.0**	3	1201472.4*	4	899305.7	4	2104437.3	1	4491873.5	449	1249556.3
FLL (kg)	31	8804.2	3	3309.0	4	17350.6*	4	7076.6	1	19235.8	449	7564.2
FSP (kg)	31	7419.8	3	1359.6	4	18523.2*	4	14598.6*	1	12587.8	373	6832.3
FDP (days)	31	2766.3	3	807.2	4	1111.3	4	3922.4	1	1166.5	373	1931.4
FCI (days)	31	7531.2	3	1148.4	4	18525.0*	4	15904.7*	1	19431.2	373	6923.0
MY/FLL (kg)	31	10.7**	3	11.8*	4	6.2	4	11.5*	1	7.1	449	4.2
MY/FCI (kg)	31	6.3**	3	15.7*	4	2.3	4	10.0**	1	0.0	373	3.7
EBV (kg)	31	494826.3**	3	415439.0	4	71441.2	4	10309.7	1	-	449	180063.5
EBE (%)	31	275.0	3	24.2	4	134.50	4	348.2	1	-	373	175.6

*Significant ($P<0.05$), **significant ($P<0.01$). FLY- first lactation yield 305-days or less; TFLY -total first lactation yield; FLL - first lactation length; FSP - first service period; FDP- first dry period; FCI- first calving interval; MY/FLL - milk yield per day of first lactation length; MY/FCI -milk yield per day of first calving interval; EBV -expected breeding value; EBE - expected breeding efficiency.

Age at first calving

The impact of AFC was non-significant on all first lactation productions and reproduction traits. The results were in agreement with the findings of Kumar (1992).

Heritability estimates

The h^2 estimates of FLY, TFLY, MY/FLL, MY/FCI and EBV were 0.41±0.13, 0.34±0.12, 0.51±0.15, 0.41±0.14 and 0.57±0.18 respectively. The results indicated that these traits were more influenced by additive genetic variability and can be improved by selection. However, the h^2 estimates of FLL, FSP, FDP, FCI and EBE were low and associated with high standard error indicating that selection had little scope and might be improved by management interventions.

It is inferred from the above findings that there is large variation in transmitting ability of sires for production traits. High levels of inbreeding were adversely affecting the production and reproduction traits therefore attempts should

Table 2. Least-squares means of performance traits in Karan Fries cattle

Traits	FLY (kg)	TFLY (kg)	FLL (day)	FSP (day)	FDP (day)	FCI (day)	MY/FLL (kg)	MY/FCI (kg)	EBV (kg)	EBE (%)
Overall means	3173±82 (490) *26*	3667±135 (490) *33*	346±108 (490) *26*	143±11 (415) *62*	75±6 (415) *64*	423±11 (415) *21*	10.6±0.2 (490) *23*	8.92±0.2 (415) *28*	3380±26 (490) *15*	88±1.3 (306) *7*
Inbreeding groups										
1	3332±70[a] (412)	3852±115[a] (412)	349±9 (412)	133±9 (350)	72±5 (350)	423±9 (350)	11.1±0.2[a] (412)	9.5±0.2[a] (350)	3417±17 (412)	89±1 (244)
2	3264±136[ab] (36)	3762±209[ab] (36)	359±16 (36)	145±18 (30)	72±9 (30)	413±17 (30)	10.7±0.4[ab] (36)	8.9±04[ab] (30)	3448±45 (36)	90±3 (36)
3	3206±168[ab] (21)	3636±268[ab] (21)	329±21 (21)	157±26 (16)	72±11 (16)	423±21 (16)	10.1±0.5[ab] (21)	8.4±0.5[ab] (16)	3355±54 (21)	91±3 (13)
4	2791±108[c] (21)	3458±280[b] (21)	348±22 (21)	157±27 (16)	85±12 (16)	432±29 (16)	9.7±0.5[c] (21)	7.8±0.5[b] (16)	3301±54 (21)	88±4 (13)
Seasons										
1	3185±108 (101)	3655±174 (101)	329±14[a] (101)	137±14[a] (89)	73±7 (89)	416±14[a] (89)	11.1±0.3 (101)	9.1±0.3 (89)	2954±58 (58)	89±3 (48)
2	3167±97 (139)	3736±157 (139)	357±12[c] (139)	153±13[b] (199)	76±6 (119)	426±12[ac] (119)	10.5±0.3 (139)	8.7±0.3 (119)	2894±44 (128)	88±2 (99)
3	3189±97 (142)	3806±157 (142)	361±12[c] (142)	168±12[b] (117)	81±6 (117)	448±13[bc] (117)	10.6±0.3 (142)	8.9±0.3 (117)	2858±48 (107)	88±2 (86)
4	3100±119 (65)	3532±189 (65)	338±15[ac] (65)	138±151[ab] (55)	71±8 (55)	415±16[ac] (55)	10.4±0.4 (65)	8.7±0.4 (55)	2954±60 (57)	90±2 (46)
5	3225±132 (43)	3655±208 (43)	343±16[ac] (43)	124±17[c] (36)	76±9 (36)	409±17[a] (36)	10.6±04 (43)	9.1±04 (35)	2959±72 (33)	90±3 (28)
Periods										
1	3017±305 (29)	3031±467 (29)	355±36 (29)	143±38[ac] (25)	91±20 (25)	431±38b[c] (25)	9.1±0.8[a] (29)	7.5±0.8[a] (25)	3352±88 (29)	91±6 (33)
2	2990±175 (148)	3002±270 (148)	316±21 (148)	99±21[a] (117)	65±11 (117)	384±21[a] (117)	9.7±0.5[ab] (148)	8.3±0.5[a] (117)	3367±53 (148)	95±3 (96)
3	3283±118 (113)	3794±182 (113)	356±14 (113)	157±15[bc] (103)	85±8 (103)	441±5[bc] (103)	10.4±0.3[ab] (113)	8.4±0.3[a] (103)	3364±36 (113)	87±2 (78)
4	3267±139 (119)	3729±214 (119)	360±17 (119)	168±17[bc] (105)	86±9 (105)	451±7[bc] (105)	10.3±0.4[ab] (119)	8.5±0.4[a] (105)	3443±51 (119)	85±3 (58)
5	3278±173 (81)	3888±268 (81)	352±20 (181)	128±22[ab] (65)	60±11 (65)	404±22[a] (65)	11.4±0.5[b] (81)	9.8±0.5[b] (65)	3443±52 (81)	89±3 (42)

Values with different superscripts within the row differed significantly. Figure in parenthesis are number of observations, value in italics is coefficient of variation in per cent.

Table 3. Regression of performance traits on inbreeding coefficient in Karan Fries cattle

Trait (s)	N	Intercept	b± SE
FLY (kg)	102	8.14	-0.0006 ± 0.0005
TFLY (kg)	102	6.48	-0.0003 ± 0.0003
FLL (days)	102	5.15	0.0036 ± 0.0045
FSP (days)	63	5.24	0.0124 ± 0.0061*
FDP (days)	63	1.67	0.0223 ± 0.0133
FCI (days)	63	1.44	0.0134 ± 0.0062*
MY/FLL (kg)	102	8.53	-0.2104 ± 0.1580
MY/FCI (kg)	63	10.98	-0.5057 ± 0.2526*
EBV (kg)	102	2600.20	-6.611 ± 19.142
EBE (%)	63	58.37	-0.173 ± 0.583

*Significant (P<0.05), b is regression coefficient, N is number of inbreds.

be made to keep inbreeding under safer limit. Coefficient of relationship may be taken in to account while using breeding bulls. Performance traits showed fluctuating trends both due to genetic and non-genetic factors, hence, require intervention both at genetic and managemental level.

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Effect of early weaning at different weaning weights on growth and feed efficiency characteristics of the Brown Swiss and Holstein Friesian calves

FEYZI UGUR[1]

Ataturk University, Faculty of Agriculture, Animals Science Department, Erzurum, Turkey

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ABSTRACT

Brown-Swiss and Holstein - Friesian calves weaned at 45 days age were separated into 2 groups (32-45 and 46-61 kg) based on their weights on 45 days age. The effect of the weight groups at 4 months old calves were significant, but, the average weights gain in the periods between birth and 6 months and feed efficiency were insignificant. The correlation between 2 weight groups at weaning and sixth months weight and between weight groups at weaning and daily weight gains between weaning and 6 month of ages were nonsignificant.

Key words: Body measurement, Calves, Early weaning, Feed efficiency, Growth

A lot of research related to feeding calves with the minimum quantity milk were conducted in dairy farms to economise their raising cost. Most of these researches were based on decreasing of the weaning age and reported successful results (Winter 1978, Yun and Chung 1985, Yanar *et al.* 1995, Ugur and Yanar 1998, Ugur 1999, Ugur *et al.* 1999). All these studies indicate that dairy calves can be weaned much earlier age and body weight.

However, giving an attention into only weaning age have not given good results, as the live weights of the calves can affect the growing performances, livability and feed consumptions of the calves in later age. Therefore in this research, based on these indications, the effect of the weight differences at weaning age on growing and feed efficiency performances of calves were investigated.

MATERIALS AND METHODS

This study was carried out at Agriculture Farm of Ataturk University, based on growth records of 24 Brown - Swiss and Holstein - Friesian calves weaned at 45 days age. Then these calves were separated into A and B groups based on the body weights at weaning time (Table 1).

Followed by colostrum for the first 3 days calves were fed whole milk constantly at 7% of their birth weight. The calves were housed in calf-sheds.

Two different calf starters (starter 1 and 2) and dried hay were used in this study. Percentages of crude protein, ether extract, crude ash, crude cellulose and dry matter for starter 1 and 2 were: 19.0, 2.12, 7.2, 8.3, 90.0 and 18.2, 2.0, 7.2, 10.0, 92.0% respectively. Starter 1 was fed from birth to 4 months of age, and starter 2 thereafter. The quantity of starters was limited as 2 kg/day. Dried hay in medium quality was offered to the calves *ad lib.* during the study, and daily consumption were recorded. The weights and body measurements were recorded at birth, weaning, 4 and 6 months of age.

Table 1. Mean weight groups at 45 days (kg)

Groups	Weight range	Mean	Birth weight
A	32-45	42.7	30.3
B	46-61	48.7	35.4

The experiment was arranged in 2 × 2 × 2 completely randomized factorial experimental design, and data were analyzed statistically using sex, breed, weight group and interactions in the model (SAS 1996).

RESULTS AND DISCUSSION

The results were presented in Tables 2-3. The differences between live weight groups (Table 1), based on weaned at 45 days age and 4 month of age (Table 2) were found statistically important ($P<0.01$). The Brown-Swiss and Holstein Friesian calves with the higher live weight at 45 days old live weight average as well.

The differences between A and B groups at 6 months age was obtained as 7.6 kg but was found statistically not significant (Table 2). Similarly, the effect of the weight groups at weaning time on daily weight gains for the periods between birth - 6 months and 4 - 6 months were found insignificant.

Present address: [1]Onsekiz Mart Universitesi Ziraat Fakultesi Zootekni Bolumu, 17100, Canakkale, Turkey.

Table 2. Least-squares means and standart error of means for weights, gains of calves

	n	Weights (kg)			Daily weight gains (kg)	
		45 days	4 months	6 months	Birth-6 months	4-6 months
Weight groups at weaning		**	**	NS	NS	NS
A	13	39.8±1.6	76.9±2.2	123.2±2.7	0.51±0.01	0.48±0.01
B	11	51.4±1.7	87.0±2.4	130.8±2.9	0.52±0.01	0.46±0.02
Sex		NS	NS	NS	NS	NS
Male (M)	12	46.6±0.16	81.4±2.2	129.3±2.1	0.52±0.01	0.46±0.01
Female (FM)	12	44.6±1.7	82.4±2.3	124.7±2.8	0.51±0.01	0.49±0.02
Breed		NS	NS	*	*	*
Brown-Swiss (BS)	14	45.5±1.5	84.9±2.1	132.6±2.5	0.54±0.01	0.51±0.01
Holstein-Friesian (HF)	10	45.7±1.8	78.9±2.4	121.4±2.9	0.49±0.01	0.43±0.02
Sex × weight groups at weaning interaction		NS	NS	NS	NS	NS
M×A	5	40.7±2.5	75.4±3.0	123.9±4.2	0.51±0.02	0.46±0.02
M×B	5	52.4±2.1	87.5±2.0	134.7±3.5	0.53±0.01	0.46±0.02
F×A	8	38.8±2.0	78.4±2.7	122.5±3.3	0.51±0.01	0.50±0.02
F×B	6	50.5±2.8	86.5±3.8	127.0±4.6	0.51±0.02	0.47±0.03
Breed × weight groups at weaning interaction		NS	NS	NS	NS	NS
BS×A	8	40.8±2.0	80.6±2.7	129.6±3.3	0.54±0.01	0.51±0.02
BS×B	6	50.1±2.3	89.3±3.2	135.5±3.9	0.55±0.02	0.51±0.02
HF×A	5	38.7±2.5	73.3±3.4	116.7±4.2	0.49±0.02	0.45±0.02
HF×B	5	52.6±2.5	84.6±3.4	126.2±4.2	0.50±0.02	0.42±0.02

**P<0.01, *P<0.05, NS nonsignificant.

Table 3. Least squares means and standart error of means for body length (BL), height at withers (HV) and heart girth (HG) of calves (cm)

	At 4 months of age			At 6 months of age		
	BL	HV	HG	BL	HV	HG
Weight groups at weaning	*	*	*	NS	*	*
A	73.7±0.09	78.5±0.9	98.2±0.9	84.6±1.1	87.8±1.0	113.7±0.9
B	76.7±0.9	82.2±1.0	101.3±1.0	87.1±1.1	91.7±1.1	114.8±1.0
Sex	NS	NS	NS	NS	NS	NS
Male (M)	75.1±0.8	80.7±0.9	100.3±0.9	85.9±1.0	90.0±1.0	115.7±0.9
Female (FM)	75.3±0.9	80.1±1.0	99.2±0.9	85.8±1.0	89.6±1.0	112.7±0.8
Breed	NS	*	NS	*	NS	NS
Brown-Swiss (BS)	76.6±0.9	82.1±0.91	100.3±0.9	87.6±0.96	90.8±1.0	115.2±0.8
Holstein-Friesian (HF)	75.7±0.9	78.6±1.04	99.1±0.9	83.9±1.11	88.8±1.2	113.4±0.9
Sex × weight groups at weaning interaction	NS	NS	NS	NS	NS	NS
M×A	73.2±1.3	78.5±1.5	99.2±1.4	84.7±1.6	88.6±1.6	115.1±1.3
M×B	74.2±1.0	78.7±1.3	97.9±1.2	84.6±1.3	87.7±1.4	112.4±1.1
F×A	77.0±1.1	82.8±1.3	101.1±1.3	87.2±1.3	91.2±1.3	116.4±1.2
F×B	76.4±1.4	81.2±1.7	100.5±1.5	87.1±1.7	91.5±1.8	113.1±1.5
Breed × weight groups at weaning interaction	NS	NS	*	NS	NS	NS
BS A	73.5±1.1	81.0±1.2	100.9±1.2	86.1±1.2	89.6±1.2	114.7±1.1
BS B	76.0±1.3	82.7±1.5	99.7±1.3	89.5±1.6	92.0±1.6	115.6±1.2
[illegible]	73 9±1.4	76.3±1.8	96.3±1.5	832±1.7	86.9±1.9	112.9±1.4
[illegible]	77 5±1.3	81.3±1 3	102.0±1.4	85.7±1.6	90.7±1.6	113.9±1.3

Dry matter consumed for per kg weight gain between birth and 6 month of age were 3.9 ± 0.10 and 3.9 ± 0.10 kg for A and B groups respectively. Similar values in the period between 45 and 120 days were recorded (3. 6 ± 0.16 and 3.7 ± 0.17 kg for A and B groups). The differences among the groups were not significant.

Weight groups at weaning had significant ($P<0.05$) effect on the body length, height at withers and heart girth at 4 months of age (Table 3). This effect was maintained at 6 months of age except for the body length. These are not contradictory to be current evaluation, however, the variations of the body measurement between groups A and B were small and these difference has little importance from breeding aspects.

According to the correlation analyses, the correlation (0.52) between weight groups at weaning and 4th months weight was found significant but other correlation values were not statistically significant.

In a similar study (Plaza and Fernandez 1999), the effect of weaning weight on growing performance of calves was important, which does not agree with our results. The rumen development of the calves with 39.8 kg live weight at 45 days age, was shown positively affected by feed consumption after weaning time. Moreover, the growing performance of the calves, weighed as 39.8 and 51.4 kg at weaned 45 days age, were the same. The study warrants more work to draw meaningful conclusion.

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Effect of chemical treatment of mustard-cake on its palatability and degradation of glucosinolates

G H PAILAN[1] and K K SINGHAL[2]

National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

Indian varieties of mustard contain appreciable level of glucosinolates, the single most important factor limiting the potentiality of this oilcake as a protein source. To reduce glucosinolates content, expeller pressed mustard-cake was treated with graded levels of 2% $CuSO_4$ and $ZnSO_4$ solutions separately with or without keeping moisture level constant and dried at 60°C for 24 hr. These differently treated mustard-cake were analysed for their total glucosinolates content, fermentability and hydrolysis end product of glucosinolates under *in vitro* condition. With the increasing quantity of 2% $CuSO_4$ solution glucosinolates content reduced gradually from 6.16±0.05 to 2.63±0.11%. Only water treatment also reduced the glucosinolates content up to 4.99±0.15%, however, $ZnSO_4$ treatment had no additional impact on glucosinolates content over water treatment. Treatment of mustard-cake with $CuSO_4$ reduced production of thiocyanate under *in vitro* rumen. However, water and $ZnSO_4$ treatment had no influence on hydrolysis of glucosinolate content. Treatment of mustard-cake with 2% $CuSO_4$ solution @ 50 ml/100 g also improved its palatability. The results indicated the possibility of interaction between Cu^{++} and glucosinolates, and judicious use of this chemical can overcome the problem related with glucosinolates in mustard-cake.

Key words: Chemical treatment, Glucosinolates, Palatability

In India mustard / rapeseed oil-cake is extensively used as a protein supplement in ruminant ration due to its availability, high crude protein content and cheaper rates. However, its glucosinolates content represents the single most important factor limiting its potential as a protein supplement. Glucosinolates content (2.54 to 7.31%) of Indian varieties of mustard/rapeseed is very high (Tyagi 1991). The pungent smell and biting taste of hydrolysis products of glucosinolates reduce the palatability of feed (Bell 1984, Hill 1991). To improve the palatability of such oil-cakes glucosinolates content is to be reduced. Water soaking is a conventional practice to improve the palatability of mustard- cake, however, in large scale particularly in feed industry such practice can not be possible. Schone *et al.* (1990) reported that plasma Cu and Zn status in pig significantly reduced by feeding glucosinolate containing rapeseed meal. However, $CuSO_4$ treated- meal maintained plasma Cu and Zn status envisaging some interaction between glucosinolate with Cu and Zn, which may reduce the glucosinolate content of such meal. The present study was therefore taken up to explore the effect of chemical treatment of mustard-cake on level and degradation of glucosinolates, and its palatability.

Present address: [1]Scientist, PAR Division, IGFRI, Jhansi, Uttar Pradesh 284 003.

[2]Principal Scientist, DCN Division.

MATERIALS AND METHODS

Treatment of cake and preparation of inoculum

Expeller pressed mustard-cake (100 g) was sprinkled and mixed with graded levels of 2% $CuSO_4$ (A) and $ZnSO_4$ (B) solution and dried at 60°C for 24 hr. The samples were also treated in same fashion maintaining constant moisture level to nullify the effect of moisture, using distilled water, irrespective of level of $CuSO_4$ (C) or $ZnSO_4$ (D) solutions (Table 1). Thus, differently treated cakes were analyzed for

Table 1. Treatments of mustard-cake with different levels of 2% $CuSO_4$ and $ZnSO_4$ solutions

Treatments	A (ml $CuSO_4$)	B (ml $ZnSO_4$)	C (ml $CuSO_4$ + ml water)	D (ml $ZnSO_4$ + ml water)
T0	0	0	0+60	0+60
T1	10	10	10+50	10+50
T2	20	20	20+40	20+40
T3	40	40	40+20	40+20
T4	50	50	50+10	50+10
T5	60	60		

total glucosinolates content using the procedure of McGhee *et al.* (1965). Fermentability and hydrolysis of glucosinolates of all the samples were studied using single stage *in vitro* technique (Tilley and Terry 1963). Rumen liquor was collected from 2 rumen fistulated crossbred bulls fed on oats fodder and concentrate mixture. Rumen liquor was drawn prior to their feeding and watering with the help of hard polythelene tube (1 m) from various sites of rumen into a pre-warmed thermos flask after straining through 4-layers of muslin cloth. Strained rumen liquor (SRL) was flushed with CO_2 to maintain the anaerobic condition.

In vitro *system*

Dried mustard-cake samples (0.5 g; treated/untreated) along with 0.25 g starch and 25 ml each of SRL and Hungate buffer were taken in 100 ml narrow mouth glass bottles fitted with rubber cork. In a separate set of bottles 0.25 g starch and 25 ml each of SRL and buffer were taken as negative control. All the bottles were sealed using aluminium caps just after transferring the inoculum and flushing with CO_2 and incubated at 39°C in a water-bath. After 24 hr of incubation, aluminium seals were broken and microbial activity was stopped using 2 drops of 1% $HgCl_2$ solution. The content of each bottles were filtered and the filtrate was used for thiocyanate estimation (Bowler 1944). The experiment was conducted in triplicate and repeated for 3 times.

Palatability of $CuSO_4$ treated mustard-cake

Crossbred (Alpine × Beetle) male kids (8) of about 4 months age and 12.5 kg body weight were divided into 2 equal groups and fed concentrate mixture 1 (group 1) and concentrate mixture 2 (group 2) along with wilted berseem as roughage source. Composition of both the concentrate mixtures is given in Table 2. The animals of respective group, already adapted to take mustard-cake and groundnut-cake containing concentrate mixture, were offered concentrate feed 5 times a day. In each group 1.0 kg concentrate mixture was offered at 9.00 hr and another 2.00 kg was offered in equal parts (0.5 kg) for 4 times at 10.00, 12.00, 14.00 and 17.00 hr (phase 1). Animals were continued on this feeding schedule for 5 days. Thereafter, untreated mustard-cake was completely replaced with 2% $CuSO_4$ (500 ml/kg) treated mustard-cake and followed the similar feeding scheduled for another 5 days (phase 2). Daily DM intake and concentrate consumption pattern of all the animals in both the phases were recorded.

Table 2. Composition of different concentrate mixture (on% DM basis)

Ingredients	Concentrate mixture 1	Concentrate mixture 2
Maize grain	50	50
Groundnut-cake (exp)	-	35
Mustard-cake (exp)	40	-
Wheat bran	7	12
Mineral mixture	2	2
Common salt	1	1
Calculated CP	19.31	19.56
Calculated TDN	75.55	74.05

Data were analysed for each parameter according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Total glucosinolates content of treated and untreated mustard-cake

Total glucosinolates content of expeller pressed mustard-cake (Table 3) decreased gradually with the increasing levels of 2% $CuSO_4$ solution and maximum reduction (57.31%) was recorded with 50 ml of 2% $CuSO_4$ solution (Table 3). The glucosinolate content reduced further in T1, T2 and T3 when the moisture level was kept constant indicating that raised moisture content might have facilitated the action of myrosinase on glucosinolates and during the course of slow drying the volatile hydrolytic products (isothiocyanates, nitriles) might have escaped. Treatment of mustard-cake with 60 ml water followed by drying at 60°C for 24 hr also reduced 18.99% glucosinolates in comparison to untreated mustard-cake (Table 3). Ludke and Schone (1988) also reported similar observations. The reduction of glucosinolates was higher than that reported by Schone *et al.* (1990) while treating rapeseed-meal under similar conditions. Glucosinolates content of treated mustard-cake was highly correlated with level of $CuSO_4$ solution used for treatment and the coefficient of correlation (r) was –0.91. In *Brassica napas* leaves, 3-indolylmethyl glucosinolate content was depressed efficiently under the influence of Cu^{++} treatment at pH 4.0 and treatment was more effective than Fe^{++} or Mn^{++} treatment due to inactivation of myrosinase (Searle *et al.* 1984). No pH adjustment was made in the present experiment as by Schone *et al.* (1990)

Table 3. Effect of different levels of 2% $CuSO_4$ and $ZnSO_4$ treatments and moisture on glucosinolates content of mustard-cake

Treatments	Glucosinolate content (%)	Treatments	Glucosinolate content (%)
Untreated (T0)	$6.16^{c}\pm0.05$	T0+ 60 ml water	$4.99^{b}\pm0.15$
10 ml $CuSO_4$ (T1)	$5.99^{c}\pm0.10$	T1+ 50 ml water	$4.37^{b}\pm0.05$
20 ml $CuSO_4$ (T2)	$5.51^{c}\pm0.27$	T2+ 40 ml water	$3.38^{a}\pm0.12$
40 ml $CuSO_4$ (T3)	$3.94^{b}\pm0.17$	T3+ 20 ml water	$3.38^{a}\pm0.12$
50 ml $CuSO_4$ (T4)	$2.63^{a}\pm0.11$	T4+ 10 ml water	$3.35^{a}\pm0.21$
60 ml $CuSO_4$ (T5)	$3.45^{ab}\pm0.35$		
10 ml $ZnSO_4$ (T1)	$5.82^{b}\pm0.10$	T1+ 50 ml water	5.05±0.10
20 ml $ZnSO_4$ (T2)	$5.69^{b}\pm0.11$	T2+ 40 ml water	5.19±0.08
40 ml $ZnSO_4$ (T3)	$5.02^{a}\pm0.06$	T3+ 20 ml water	5.22±0.08
50 ml $ZnSO_4$ (T4)	$4.91^{a}\pm0.06$	T4+ 10 ml water	5.02±0.13
60 ml $ZnSO_4$ (T5)	$4.94^{a}\pm0.10$		

abc: Values bearing different superscripts in a column for individual treatment ($CuSO_4$ or $ZnSO_4$) differ significantly ($P<0.01$)

but the reduction in glucosinolates content suggested either Cu^{++} interaction with glucosinolates or depressed myrosinase enzymatic system (Youngs *et al.* 1971). Such interaction (s) needs thorough investigation.

Treatment of mustard-cake with different levels (10 to 60 ml) of $ZnSO_4$ solution reduced glucosinolates content (Table 3). However, when moisture level was kept constant with varying levels of 2% $ZnSO_4$ solution, glucosinolates content of mustard-cake did not reduce. This result indicated that in mustard-cake the apparent reduction of glucosinolates content due to $ZnSO_4$ treatment may be attributed to the moisture. Similar to this observation, Ludke and Schone (1988) also reported that treatment of mustard-cake with different levels of 2% $ZnSO_4$ solution did not influence its glucosinolates content.

Concentration of thiocyanates in incubation medium

Thiocyanate concentration in incubation medium after 24 hr of incubation of untreated or differently treated mustard-cake samples under *in vitro* system are presented in Table 4. The concentration in untreated cake was 4.13±0.07 mg/100 ml and the value was slightly higher than the level (3.3 mg/100 ml) reported by Saha and Singhal (1993), which may be attributed to the higher glucosinolates content of mustard-cake (6.16%) used in this study than that used in earlier study (3.62%). Glucosinolates content of mustard-cake is hydrolysed into thiocyanate, isothiocyanate, nitrile, glucose and sulphate ions. However, in neutral pH, thiocyanate is the main metabolite and possibility of nitrile production is remote as nitrile is the main product at lower pH (Larsen 1981). $CuSO_4$ treatment reduced the hydrolysis of glucosinolates into thiocyanate which may be due to the binding of Cu^+ with glucosinolates or inactivation of myrosinase enzyme present in oil-cake. Pigs fed on $CuSO_4$ treated repeseed meal maintained the plasma T_3 and T_4 levels, which also indirectly focused that $CuSO_4$ treatment may reduce the thiocyanate production (Ludke and Schone 1988). However, this possibility has to be explored further. $ZnSO_4$ treatment had no impact on the hydrolysis of glucosinolates into thiocyanate.

Table 4. Effect of different levels of 2% $CuSO_4$ and $ZnSO_4$ treatment and moisture on thiocyanate production (mg/100 ml inoculum) in *in vitro* rumen fermentation

Treatments	SCN^- level	Treatments	SCN^- level
Untreated	4.13±0.07	60 ml water	4.04±0.26
20 ml $CuSO_4$	3.23±0.21	10 ml $CuSO_4$+ 50 ml water	2.97±0.45
50 ml $CuSO_4$	2.98±0.30	50 ml $CuSO_4$+ 10 ml water	2.09±0.45
20 ml $ZnSO_4$	3.99±0.06	10 ml $ZnSO_4$+ 50 ml water	4.09±0.07
50 ml $ZnSO_4$	3.81±0.50	50 ml $ZnSO_4$+ 10 ml water	3.79±0.23

Similarly water-treated mustard-cake showed similar level of thiocyanate in incubation medium as observed in untreated mustard-cake, which showed that simple water treatment has no effect on hydrolysis of glucosinolates during rumen fermentation. Enhancing the moisture level of $CuSO_4$-treated cake to the level of water-treated cake (50 ml $CuSO_4$ + 10 ml water) was most effective for hydrolysis of glucosinolates into thiocyanate.

Palatability of $CuSO_4$ treated mustard-cake

Average daily DM intake through concentrate mixture in group 1 and group 2 was 0.33±0.03 and 0.36±0.02 kg, respectively in phase 1. However, in phase 2, when $CuSO_4$-treated mustard-cake replaced the untreated mustard-cake in the concentrate mixture of group 2, daily concentrate mixture intake was 0.40±0.02 and 0.34±0.06 kg in group 1 and 2 respectively. Intake of concentrate mixture was similar in groundnut-cake and untreated mustard-cake supplemented groups, however, feeding of treated mustard-cake improved the intake of concentrate feed by 21.78% without affecting the total DM intake (Table 5). Kids fed on untreated mustard-cake containing concentrate mixture (MC) used to consume the concentrate mixture slowly (Fig.1) and a sizable amount of concentrate mixture used to remain as leftover even after 24 hr of feeding. However, treated mustard- cake (TMC) supplementation not only improved the rate of concentrate intake but the leftover was only about 15% as recorded in groundnut-cake supplemented concentrate mixture (GNC). Dry matter intake through roughage was similar in both the groups during phase 1 and in phase 2 (Table 5). Total DM

Table 5 Palatability of $CuSO_4$ treated mustard cake containing concentrate mixture in kids

Parameter	Group 1	Group 2
Phase I		
DMI through concentrate (kg/d)	0.33±0.03	0.36±0.02
DMI through roughage (kg/d)	0.23±0.01	0.23±0.02
Total DMI (kg/d)	0.56±0.04	0.59±0.04
Phase II		
DMI through concentrate (kg/d)	0.40±0.02	0.34±0.06
DMI through roughage (kg/d)	0.18±0.02	0.17±0.02
Total DMI (kg/d)	0.58±0.02	0.51±0.05

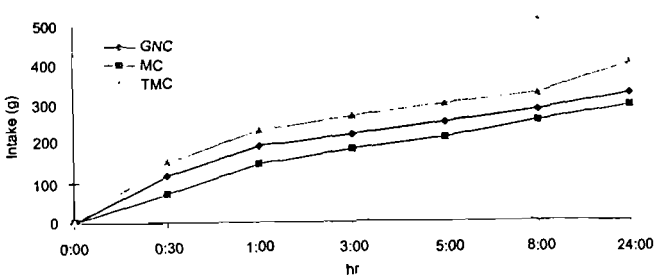

Fig. 1. Consumption pattern of different concentrate mixtures in kids.

intake per 100 kg live weight was about 4.5 kg and remained uninfluenced by feeding of treated mustard-cake.

It can be concluded that $CuSO_4$ treatment is quite effective for reducing glucosinolate content of mustard-cake besides reducing its hydrolysis into thiocyanate, however, $ZnSO_4$ treatment did not reveal any benefit. Palatability of high glucosinolates containing mustard-cake can be improved by $CuSO_4$ treatment.

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Carbohydrate and nitrogen fractionation of certain feeds and their utilization in growing buffaloes fed on total mixed ration

JEYA PRAKASH R[1], S S KUNDU[2] and M M DAS[3]

National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

Twelve Murrah reverine buffalo calves (age 18-30 months, av. b. wt. 264.6 kg) were fed complete diet comprised 59.3 parts (DM basis) wheat straw, 22.30 parts berseem, 10.04 parts mustard-cake and 8.26 parts of wheat bran. All the ingredients of complete diet were analysed for proximate and chemical composition as per cornell net carbohydrate and protein system (CNCPS). In complete feed NPN, ADFIN (PC), true protein (PB2) contents were 7.85, 12.30 and 42.31% whereas available fibre (CB2), unavailable fibre (CC) and starch were 53.90, 23.43, and 1.96% respectively. Peptide fraction (RDPEP, 310.16g) and fibre fraction (RDCB2, 1469. 30g) degraded maximally in the rumen. The degradable fraction of protein and carbohydrates predicted from CNCPS model and from nylon bag study were 352.18 vs 268.84 g/day and 2101.62 vs 1297.27 g/day. Digestibility coefficients of DM, OM, CP, NDF and ADF were 56.34, 60.16, 50.56, 50.66, 47.94 and 53.75%. TDN (3.06 kg/d) and metabolizable protein (MP, 328.43 g/d) as predicted from the model were comparable with the TDN availability, CP (580.80 g/day) and DCP (294.02 g/day) intake from the metabolism trial. The result of the present study indicated that CNCPS model can be applied for the prediction of nutrient availability from feeds and forages in total mixed ration feeding system, however, the model needs to be tested further on larger scale in Indian conditions.

Key words: Buffalo calves, Cornell Net Carbohydrate and Protein System, Metabolizable protein, Total mixed ration, Total digestible nutrient

Accurate prediction of forage nutritive values and performance of animals requires accurate accounting for factors that influence animal requirements and feed stuff utilization. Conventional proximate analysis and cell wall determinations meet these criteria only partially. Since carbohydrate fractions such as sugar, starch, non starch polysaccharides and other structural carbohydrates and also fractions of crude protein such as NPN, soluble peptides, soluble true protein, insoluble protein are utilized by different rumen microbes and animals differently. The degradation rates, as well as their passage rates which are very important determinants of net energy and protein, also varies according to fractions. The cornell net carbohydrate and protein system i.e. (CNCPS) is an application model that uses a combination of mechanistic and empirical approaches and also accounts for the facts of variation in animal factors (Fox *et al.* 1995). This model provides more precise and accurate data on feeding values of feeds, forages and animal requirements than NRC (1989). In present study, the feeds used, are analysed as per CNCPS model and the total mixed ration fed to buffaloes to understand and evaluate the applicability of the model under tropical Indian conditions.

MATERIALS AND METHODS

Twelve Murrah reverine buffalo calves (average bwt. 264.62 kg) of 1.5 to 2.5 years of age were selected from herd maintained at the National Dairy Research Institute, Karnal, Haryana. All the animals were shifted to preliminary feeding of total mixed ration comprised 59.3 parts wheat straw, 22.30 parts berseem, 10.04 parts mustard-cake and 8.26 parts of wheat bran which was fortified with mineral mixture 30 g and salt 20 g for 21 days. After the preliminary feeding of 3 weeks, daily dry matter intake of animals were recorded for 6 weeks, and then a conventional metabolism trial of 7 days collection period was conducted (Schneider and Flatt 1975). All the feeds were analysed for proximate principles (AOAC 1995), cell wall contents (Van Soest 1991), protein fractions (Licitra *et al.* 1996), starch (AOAC 1995) and other carbohydrate fractions as per Snifen *et al.* (1992). The data on feed fractions were fitted in CNCPS model (Russel *et al.* 1992, Snifen *et al.* 1992). Nylon bag degradability of these feeds were determined as per the models suggested by Orskov and Mac Donald (1979).

Present address: [1]Veterinary Officer, Maharashtra.

[2]Head Scientist, Plant Animal Relationship Division, IGFRI, Jhansi.

RESULTS AND DISCUSSION

The chemical composition data of different ingredients of total mixed ration (Table 1) are comparable to available literature (Ranjhan 1991). Acid detergent insoluble protein (ADFIP) fraction of CP (Table 2) was higher in wheat straw and berseem and indicates the lower availability of N as protein is associated with lignin and or maillard products that are highly resistant to microbial enzymes (Krishnamoorthy *et al.* 1982, 1983). NPN per cent of soluble CP was highest in wheat bran and lowest in wheat straw. Neutral detergent fibre (NDF) as percentage of DM was found highest in wheat straw followed by berseem and wheat bran, whereas lignin as per cent of NDF was highest in mustard-cake. The higher NDF content of wheat straw was largely due to higher cellulose content. Non structural carbohydrates were maximum in wheat bran of which starch was only a small fraction in all feeds which ranged between 1.78 to 14.5%.

All the values of different protein fractions are in good agreement with the earlier report (Sharma and Singh 1997). Different protein and carbohydrate fractions as per CNCPS model, of total mixed ration and their degradation in rumen (Table 3) indicated that NPN component was lowest (7.85%) followed by unavailable ADF bound protein (PC, 12.30%), whereas the true protein content was highest (PB2, 42.31). Among the different carbohydrate fractions, the available fibre fractions was maximum (CB2, 53.9%), whereas non available carbohydrate i.e. CC was 27.43% which was bound with lignin and starch content (CBI) was only 1.96%. In rumen, peptide fraction (RDPEP) appeared maximally degraded, whereas least degradation was observed with slowly degradable fraction (PB2). Since fibre content was more in the feed, the degradable fibre content was also higher (RDCB2, 1469.3g/d). The different undegradable fractions of protein and carbohydrates indicates that B3 fraction (REPB3, 74.48g) was maximum in protein, whereas unavailable bound carbohydrate (RECC, 990.36 g) was maximum from carbohydrate. Dry matter degradability of different ingredients of total mixed ration as determined by nylon bag study (Table 4) showed

Table 1. Various proximate principles and cell wall constituents of the feeds offered (% DM basis)

Particulars	DM	CP	CF	EE	NFE	ASH	NDF	ADF	Cellulose	Hemi-cellulose	Lignin
Wheat straw	88.03	3.39	32.51	0.55	51.58	11.97	78.59	50.82	34.86	27.77	10.56
Wheat bran	92.82	16.17	9.18	2.74	64.73	7.18	47.29	14.36	8.57	32.94	5.25
Mustard-cake	91.45	37.14	9.19	1.63	43.49	8.55	26.86	15.90	7.90	10.96	6.32
Berseem	86.30	18.70	28.59	2.75	35.99	13.77	52.51	25.28	14.96	27.23	6.00

Table 2. Carbohydrate and protein fractions, fat and ash in feed ingredients, and total mixed ration

Feed stuff	NDF% of DM	Lignin% of NDF	NSC% of DM	Starch% of NSC	CP% of DM	Soluble% of CP	ADFIP% of CP	NPN% of SP	NDFIP% of CP	Fat% of DM	Ash% of DM
Wheat straw	78.59	13.44	7.66	14.50	3.39	12.09	13.42	17.13	63.70	0.55	11.97
Berseem	52.51	11.43	20.44	6.94	18.90	14.80	14.40	34.52	38.35	1.63	13.77
Wheat bran	47.29	11.10	29.25	1.78	16.17	40.60	3.25	41.13	22.41	3.74	7.18
Mustard cake	26.86	23.52	27.27	7.54	37.14	57.20	2.07	19.13	6.92	2.75	8.55
Total mix feed	65.03	13.35	10.49	11.07	11.27	29.23	12.30	26.85	28.92	1.27	11.51

ADFIP, Acid detergent insoluble protein; NDFIP, neutral detergent insoluble protein.

Table 3. Proteins and carbohydrate fractions of total mixed ration (4.75 kg/d) and their degradation pattern in rumen

Attributes		Rumen degradable fractions (g/d)	Ruminally escaped fractions (g/d)
Protein fractions, % of CP			
NPN (PA)	7.85	42.02	-
Rapidly degradable protein (PB_1)	21.34	112.78	1.45
Int. degradable protein (PB_2)	42.31	189.70	36.80
Slowly degradable protein (PB_3)	16.20	7.68	74.48
Bound protein (PC)	12.30	-	68.65
Rumen degradable peptides (RDPEP)	-	310.16	-
Carbohydrate fractions (% CHO)			
CNSC	18.61	-	-
Sugar (CA)	16.71	566.70	4.40
Starch (CB_1)	1.96	65.62	5.09
Available fibre (CB_2)	53.90	1469.30	36.80
Unavailable fibre (CC)	27.43	-	990.36

Passage rate for forages (1.86%/hr), concentrate (2.27%/hr), total mixed feed (1.94%/hr).

Table 4. Dry matter degradability of the feed ingredients

Feed	DM left after incubation (%)	a (%)	b (%)	c (fraction/hr)	Potential degradability (a+b)	Residual S.D. (%)	Effective degradability* (%)
Mustard-cake	29.40 (24 hr)	39.83	60.17	0.03	100.00	2.21	61.80
Wheat bran	43.09 (24 hr)	14.34	44.94	0.13	59.28	2.54	47.10
Berseem	24.59 (48 hr)	7.41	68.13	0.09	75.54	2.06	52.50
Wheat straw	55.89 (48 hr)	-2.29	47.87	0.07	45.58	2.94	26.20

*At K=0.05 per cent per hr.

that soluble fraction of the dry matter (a) in mustard-cake was maximum whereas it was lowest in wheat straw. Potentially degradable fraction (b) of feeds was found maximum in berseem followed by mustard-cake, wheat straw and wheat bran. The potential as well as effective degradability at the flow rate of 0.05%/hr was higher for mustard-cake and lowest in wheat straw and are within the normal range of publish data (Walli 1995, 1998). The degradable fractions of protein (352.18g/day) and carbohydrate (210.62g/day) as predicted from CNCPS model were higher than the calculated values (268.84g/day and 1297.27 g/day) whereas undegradadable fractions of protein (181.38 vs 266.48g/day) and carbohydrate (1036.65.vs 2320.56g/day) follow the reverse trend. The bacterial synthesis as per CNCPS model (Table 5) reveal that bacterial true protein (REBTP) synthesis was maximum (230.6g) followed by bacterial carbohydrate (REBCHO, 218.80g), bacterial cell wall protein (REBCW, 95.86g), bacterial fat (REFAT, 73.62g) and the unavailable nucleic acid fraction (REBNA, 57.52g). The completely digestible fractions available for absorption from the intestine was less from feed protein (DIGFP, 97.83g) than through bacteria (DIGBTP, 230.60g). Carbohydrates available as VFA was maximum and the fat availability from feed and bacteria was comparable. Application of feed fraction in CNCPS model gave values of structural carbohydrate fermenting bacteria production as 420.2 g/day sand non structural carbohydrate fermenting bacteria as 193.31 g/day. As the feed in present study was predominantly roughage based, the structural carbohydrate fermenting bacterial synthesis was more. Fecal losses from feed (Table 6) appeared maximum through carbohydrate (FEFC, 998.74g) and minimum through fat (FEFAT, 4.71g), whereas from bacteria maximum losses was observed with protein fraction (FEBCW, 95.86g) followed by bacterial ash (FEBASH, 29.99g), bacterial carbohydrate (FEBC, 10.96g) and bacterial fat (FEBF, 3.68g). As this model accounts for endogenous losses, the data have also been predicted for the same and the values were (FE ENG P, 20.72g; FE ENG F, 1.03g; FE ENG A, 6.47g). Total dry matter loss through faeces as predicted from the model was about 1.79 kg. DDMI, DOMI, CP, DCP intake as determined from the conventional metabolism trial were 2.67 ± 0.05, 2.55 ± 0.04, 580.88 ± 7.15 and 294.03 ± 9.37 g/day,

Table 5. Nutrients availability from bacteria and intestine

Attributes	(g/day)
Bacteria	
REBTP	230.60
REBCW	95.86
REBNA	57.52
REBCHO	218.80
REFAT	73.62
REBASH	29.99
Intestine	
Protein	
DIGFP	97.83
DIGBTP	230.60
DIGBNA	57.52
DIGP	385.95
Carbohydrate	
VFA	2102.20
DIGFC	15.83
DIGBC	207.86
DIGC	2325.90
Fat	
DIGFFAT	57.31
DIGBF	69.95
DIGF	127.26

SCBACT (420.20g/day), NSCBACT (193.31 g/day), RAN. (152.20).

respectively, whereas, N intake/kg DOMI was 36.53 ± 0.04g, which appeared to be comparable and marginally on higher side than data reported in literature (ARC 1980). TDN (kg/day) and water intake/100 kg bwt during metabolism trial were 2.96 ± 0.06 kg and 7.82 ± 0.49 l, respectively. The data on digestibility coefficient of total mixed ration indicated that DM and OM digestibility were 56.34 and 60.16%, respectively. The predicted CP, EF digestibility (65.85, 96.43%) from the model were observed to be higher than the values (50.56, 79.69%) determined through trial. The digestibility coefficients of CF, NDF, ADF, hemicellulose were 57.52, 50.66, 47.94 and 53.75% respectively. TDN availability (3.06 kg/day) from the given feed as predicted from the model was comparable with the average TDN availability (2.96 kg/day) through the conventional digestibility trial. Metabolizable protein availability (MP)

Table 6. Nutrient utilization from total mixed ration by CNCP system

Faecal losses from feed							
Protein		Carbohydrate		Fat		Ash	
Fractions	(g/day)	Fractions	(g/day)	Fractions	(g/day)	Fractions	(g/day)
$FEPB_3$	14.90	FECB1	1.02	FEFAT	4.71	FEFA	543.89
FEPC	68.65	FECB2	7.36				
FEFP	83.55	FECC	990.36				
		FEFC	998.74				
Faecal losses from bacteria							
FEBCW	95.86	FEBC	10.96	FEBF	3.68	FEBASH	29.99
FE ENG P	20.72	-	-	FE ENG F	1.03	FE ENG A	6.47
Total faecal losses							
FEPROT	200.13	FECHO	1009.68	FEFAT	471	FEASH	580.35
TDN							
TDNAPP	3057.68						
ME							
MEa (Mcal/d)	11.05						
MEc (Mcal/kg DM)	2326.00						
MP	328.43						

from the given feed was 328.43g/day and was comparable with the conventional calculations of CP and DCP intake of 580.8 and 294.02g/day ME intake (Mcal/d) was 8.80 ± 0.18 and was 3% higher than the maintenance requirement (Kearl 1982). The excess intake of metabolizable protein (83.34g/day) than the maintenance requirements (Fox *et al.* 1992) can support growth of 51.2g/day (McDonald 1995) but in the present study it was reflected in the average daily gain of 363.2 ± 36.44g/day. It can be concluded that CNCPS model although appeared to over estimates the degradability of protein and carbohydrate fractions of feed in rumen but the predicted nutrient availability in terms of TDN and Metabolizable protein was comparable with the calculated values in the present study. Thus CNCPS model can possibly be applied for the prediction of nutrient availability from feeds and forages in different feeding system, however, more data under Indian condition could be generated before applying the same.

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Effects of trace elements supplementation in commercially reared dairy cows of different lactation in relation to mineral metabolism

S HALDAR[1], T K GHOSH[2] and N PAL[3]

West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal

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ABSTRACT

An on-farm trial was conducted with crossbred cows of 2nd and 3rd lactation to study the effects of supplemental Cu, Mn and Zn on utilization efficiency of major (Ca and P) and trace (Cu, Fe, Mn and Zn) elements. The animals received 3 treatments in a switchover fashion to receive Cu, Mn and Zn at approximately 120% (T_1), 100% (T_2) and 140% (T_3) of the respective planes of requirement (NRC 1989). Intakes of Ca, P and Fe were above the level of requirements in all the treatment groups irrespective of lactation number due perhaps to the fairly high concentrations of these elements in diet. Apparent absorption (%) of Cu, Mn and Zn were higher ($P < 0.01$) in T_2 where the intake was minimum ($P < 0.01$). Though intakes of these trace elements were higher ($P < 0.01$) in T_3 the respective apparent absorption did not vary much between T_1 and T_3. The pattern of absorption, however, remained unaffected with the lactation number. Plasma concentrations of both major (Ca and P) and trace (Cu, Fe, Mn and Zn) elements were not affected by treatment or by lactation number and no interaction could be observed between them. Plasma concentrations of all these minerals were above the respective minimum critical levels. Milk mineral concentrations were also within the normal ranges of variation with little effects of either treatments or lactation numbers. It was concluded that there is need to optimize the dietary levels of various major and trace elements in order to maximize their efficiency of utilization in lactating cows.

Key words: Cows, Mineral supplements, Nutrition, Trace elements

Strategic supplementation of ruminant diets, particularly with specific major and trace elements, rectifies the nutrient imbalances and induces an optimum rumen condition for efficient microbial fermentation (Singh and Chabra 1996). To build up an adequate storage of metabolically important mineral elements in the body to cope up with any sudden rise in requirements, the diet should contain optimum quantities of all these micro-nutrients (McDowell *et al.* 1984). However, optimizing dietary levels of different major and trace elements through supplementation needs cautious approach as interactions amongst these mineral elements interfere with their absorption and metabolism leading to secondary deficiency (Olson *et al.* 1999). Sarkar (2000) and Ghosh *et al.* (2001a) reported that supplemental major and trace elements increased the efficiency of utilization of organic nutrients in lactating dairy cows but the utilization of the respective inorganic nutrients declined when the dietary intakes increased beyond certain limits. Lactation number is yet another factor, which may affect the serum micronutrient concentration in lactating dairy animals (Rajora *et al.* 1997). Lactation number governs the production needs of animals, which in turn may bring about a change in the pattern of absorption of these mineral elements from the gut. The present investigation, which was carried out in an on-farm set up, was designed to study the metabolism of certain major and trace elements in crossbred dairy cattle of second and third lactation supplemented with Cu, Mn and Zn at various dose levels.

Present address: [1]Lecturer, [2]Professor, [3]M.V.Sc. Student, Department of Animal Nutrition, Faculty of Veterinary Science.

MATERIALS AND METHODS

Animals and feeding

To ascertain the effects of supplemental Cu, Mn and Zn at different dose levels, 8 crossbred (Holstein 50: Hariana 50) lactating cows of second and third lactation were selected from a commercially maintained dairy herd near Kolkata. These animals were divided into 2 groups, each comprising of 4 animals, according to the lactation number, viz. L-2 (comprised only second lactation animals) and L-3 (comprised only third lactation animals). At the beginning of the experiment the cows were 80 – 90 days post partum and the average milk yields were 9.3 ± 0.81 and 8.9 ± 0.84 kg day^{-1} animal^{-1} in L-2 and L-3 respectively.

Feeding regime

The normal feeding regime of the animals in the on-farm

set up was not altered. Each of the experimental animals was offered with 2 kg concentrate mixture as maintenance allowance and the same quantity as production allowance @ 0.5 kg per kg milk produced. The concentrate mixture was comprised of (kg per 100 kg) corn soya blend (25), bulgar wheat (25), wheat bran (23), mustard-cake (15), *mung chunni* (10) and salt (2). The total amount of the concentrate feed was divided into 2 equal proportions and was supplied to the animals at 5: 30 hr and 14: 30 hr during milking. Roughage fraction was supplied through paddy straw (4 kg animal^{-1}day^{-1} supplied after the evening milking) and hybrid Napier grass (12 kg animal^{-1}day^{-1} supplied at around 11.00 hr).

Details of experiment

The experiment was designed in such a way that every animal of both L-2 and L-3 got exposed to 3 different treatments, viz. T_1, T_2 and T_3 in a phase wise manner to avoid animal variations. In the first phase the concentrate mixture supplied to the animals was fortified with a mineral mixture (Table 1) that, besides other major and trace elements, contained 1500 ppm Cu, 2000 ppm Mn and 1000 ppm Zn. This mineral mixture was fed to the animals @ 15 g day^{-1} for 60 days (T1). In the second phase of the experiment supplemental mineral mixture was totally withheld from the diet and the animals did not receive any mineral elements but for those present naturally in the feeds and fodder (T_2). The duration of T_2 was 60 days after which the animals were switched over to the third phase of the experiment (T_3). In this phase the feeding regime was identical to that followed during T_2; however, each of the experimental animals was supplemented daily with Cu (30 mg), Mn (160 mg) and Zn (170 mg) in the form of gelatinized capsule. The dose levels of Cu, Mn and Zn in T_3 were fixed in such a way that the dietary levels of these trace elements increased by 40% of their plane of requirements stated by NRC (1989). The duration of this phase was also 60 days. Digestibility trials of 7 days collection was conducted in each phase at the end of 50 days feeding. The data obtained in T_3 were compared with that of T_1 and T_2 to determine the effects of different dose levels of Cu, Mn and Zn on utilization efficiency of different inorganic nutrients in animals of second and third lactation.

Table 1. Chemical composition (DM basis) of feeds and fodder

	Paddy straw	Hybrid napier	Concentrate mixture*
Proximate composition (%)			
DM	88.5 ± 0.45	19.7 ± 3.14	91.8 ± 0.81
OM	85.6 ± 2.10	84.4 ± 0.43	92.3 ± 0.26
OP	3.4 ± 0.13	14.5 ± 1.19	17.8 ± 0.68
Ether extract	1.5 ± 0.33	1.7 ± 0.13	2.4 ± 0.14
Crude fibre	34.1 ± 1.57	25.3 ± 1.78	8.9 ± 0.15
N-free extract	47.2 ± 1.91	42.8 ± 2.91	63.1 ± 0.78
*Major and trace elements***			
Ca%	0.37 ± 0.02	0.60 ± 0.06	1.95 ± 0.04
P%	0.15 ± 0.61	0.30 ± 0.01	0.99 ± 0.04
Cu ppm	9.0 ± 0.76	12.0 ± 0.73	9.5 ± 0.92
Fe ppm	250.8 ± 1.59	219.8 ± 2.14	251.9 ± 3.28
Mn ppm	12.0 ± 1.95	35.0 ± 2.14	61.0 ± 1.53
Zn ppm	25.0 ± 1.83	60.0 ± 5.5	45.0 ± 1.18

*In T_1 concentrate mixture was fortified with the following mineral mixture: Ca 500 g kg^{-1}, P 180 g kg^{-1}, Mg 1000 PPM, Cu 1500 PPM, Co 160 PPM, Iodine (KI) 200 PPM, Mn 2000 PPM, Fe 3000 PPM, Zn 1000 PPM. **critical levels of mineral elements in feeds and fodder: Ca 0.30%, P 0.25% (Knebusch *et al.* 1986), Fe 50 PPM, Cu 8 PPM, Mn 40 PPM, Zn 40 PPM (Rojas *et al.* 1993).

The DM intake and milk yields of individual animals were recorded everyday throughout the experiment (180 days). Body weight was recorded at every fortnightly intervals for 2 consecutive days. Milk samples were collected at every 20 days intervals from each individual animals and were analysed for major (Ca and P) and trace (Fe, Cu, Mn and Zn) elements. Blood samples were collected aseptically from the jugular vein of each animal in a heparinized vial at the beginning of the experiment and subsequently at monthly intervals. Plasma, separated by centrifuging the blood sample at 3 000 rpm in Remi Research Centrifuge (R – 24) for 20 min, was stored at –20°C for subsequent analysis of major (Ca and P) and trace (Cu, Fe, Mn and Zn) elements.

Analytical techniques

Feeds, fodder and feces samples collected during the digestibility trials were analysed for organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF) and N-free extract as per the method of A O A C (1980). Ca and trace elements viz. Fe, Cu, Mn and Zn in feeds and fodder were analysed by atomic absorption spectrophotometer (Perkin Elmer A Analyst 100) following wet oxidation of 0.5 g of sample in triacid mixture of concentrated H_2SO_4, HNO_3 and $HClO_3$ (6: 4: 1 v / v). Feed P was determined colorimetrically as per the method described by Talapatra *et al.* (1940). Ca and trace element (Fe, Cu, Mn and Zn) concentrations of milk and blood were determined by atomic absorption spectrophotometer (Arenza *et al.* 1977). P in milk and plasma was determined as per the method described by Fiske and Subba Row (1925).

Statistical analysis

The pattern of intake and utilization of various organic nutrients as well as the yield of milk and milk constituents were compared between the treatments following two-way analysis of variance (with interaction) technique. The data were analysed by MS-Excel Software package to determine the effects of different treatments, lactation number and the interaction between treatments and lactation number on the respective parameters. The statistical model used is as follows:

$$y_{ijk} = \mu + l_i + a_{ij} + t_k + (lt)_{ik} + e_{ijk}$$

Where y_{ijk} = observation for the kth treatment on the jth animal within the ith lactation

μ = overall mean

l_i = effect of l th lactation.
a_{ij} = effect of j th animal within the ith lactation
t_k = effect of k th treatment
$(lt)_{ik}$ = interaction effect for lactation and treatment
e_{ijk} = random error (normally and independently distributed).

RESULTS AND DISCUSSION

Chemical composition of diet

The proximate composition of concentrate mixture, paddy straw and hybrid Napier was within the normal ranges of variations (Table 1). However, the DM of hybrid napier varied widely from 12.3 to 27.6% and this might be due to seasonal effect. The initial samples were collected in wet winter months (late January – early February) while the latter samples were drawn during the dry summer (May – June). The concentrate mixture used in this experiment had 17.8% CP and therefore can be termed as a balanced concentrate mixture.

Concentrations of Ca in paddy straw, hybrid Napier and concentrate mixture were much above the respective minimum critical levels (Knebusch *et al.* 1986) though that of P in paddy straw (0.15 ± 0.01%) was deficient. P in hybrid Napier (0.30 ± 0.01%) and concentrate mixture (0.99 ± 0.04%) were within the normal range. The Ca: P ratios in the concentrate (1.86 to 2.07), hybrid Napier (1.51 to 2.09) and paddy straw (2.27 to 2.58) indicate fairly even distribution of Ca and P in the diet. Fe in all the roughage and concentrate ingredients were much higher than the minimum critical value of 50.0 ppm (Rojas *et al.* 1993) as has been reported earlier also by Das *et al.* (2002). Mn was deficient in both paddy straw (15.4 ± 1.95 ppm) and hybrid Napier (34.8 ± 0.50 ppm) while a marginal deficiency of Zn was observed in paddy straw (28.1 ± 1.93 ppm).

Intake of inorganic nutrients

Ca and P: Ca and P intakes (g day^{-1} $animal^{-1}$) were higher ($P < 0.01$) during T_1 (141.4 ± 4.25 and 73.0 ± 3.19 respectively) vis-a-vis that in T_2 (111.6 ± 4.79 and 55.4 ± 2.51 respectively) and T_3 (108.8 ± 4.48 and 58.2 ± 1.75 respectively). It is important to note that the diets consumed by the animals during T_2 and T_3 did not have any exogenous Ca and P supplement. Moreover, the total DMI in T_1 was higher ($P < 0.01$) than T_2 and T_3 (12.4 ± 0.27, 10.1 ± 0.30 and 9.8 ± 0.33 Kg $animal^{-1}$ respectively) because as per the feeding regime of the farm the amount of concentrate supplied to the animals decreased consequently with a decline in milk production. These 2 factors, coupled together, resulted in the higher intake of Ca and P in T_1 (Table 2). The intake of Ca in all the 3 phases of the experiment was much higher than the NRC (1989) recommended dietary level of 0.43 to 0.60% (1.14 ± 0.01, 1.10 ± 0.01 and 1.11 ± 0.02%, respectively, in T_1, T_2 and T_3). Intake of P (0.59 ± 0.01, 0.56 ± 0.01 and 0.60 ± 0.01% in T_1, T_2 and T_3 respectively) was also above the requirement (0.31 to 0.40%) and these might be due to the fairly high concentrations of Ca and P in almost all the dietary ingredients. Lall et al. (1994) and Ghosh et al. (2001b) also reported that supplementation of ruminant diets with major and trace elements resulted in higher intake of the respective minerals by animals. However, lactation number did not exert any effect on intake of Ca and P. While Ca intake in L-3 was

Table 2. Intake and apparent absorption of major (Ca and P) and trace (Cu, Mn, Zn and Fe) elements

		T_1 (60 days)		T_2 (60 days)		T_3 (60 days)		
		L-2	L-3	L-2	L-3	L-2	L-3	C. D
Ca	Dietary intake g day^{-1}	142.1 ± 6.39^a	140.7 ± 6.57^a	109.1 ± 8.23^b	114.2 ± 5.93^b	107.4 ± 9.34^b	110.1 ± 7.20^b	7.75**
	Outgo (% of intake)	58.9 ± 1.94	58.1 ± 1.86	54.5 ± 4.88	55.1 ± 4.10	53.1 ± 5.30	54.3 ± 4.59	
	Apparent absorption (%)	41.1 ± 1.46^b	41.9 ± 1.48^b	45.5 ± 0.65^a	44.9 ± 1.18^a	46.9 ± 0.48^a	45.7±0.92^a	2.67**
P	Dietary intake g day^{-1}	73.4 ± 4.76^a	72.6 ± 4.99^a	54.3 ± 4.45^b	56.5 ± 2.99^b	58.3 ± 3.20^b	58.1 ± 1.97^b	4.79**
	Outgo (% of intake)	46.5 ± 3.49	46.1 ± 3.98	39.2 ± 2.60	39.8 ± 1.81	40.1 ± 1.93	40.3 ± 1.33	
	Apparent absorption (%)	53.5 ± 1.82^b	53.9 ± 2.32^b	60.8 ± 1.44^a	60.2 ± 1.44^a	59.9 ± 0.84^a	59.7 ± 1.31^a	1.72**
Cu	Dietary intake ppm	11.9 ± 0.05^b	11.8 ± 0.08^b	10.1 ± 0.02^c	10.0 ± 0.02^c	13.8 ± 0.01^a	13.8 ± 0.03^a	1.50**
	Outgo (% of intake)	86.4 ± 0.50	86.9 ± 0.82	81.3 ± 0.25	81.8 ± 1.03	85.1 ± 0.25	85.4 ± 0.48	
	Apparent absorption (%)	13.6 ± 0.96^b	13.1 ± 1.97^b	18.7 ± 0.61^a	18.2 ± 1.08^a	14.9 ± 0.65^b	14.6 ± 1.05^b	2.15**
Mn	Dietary intake ppm	43.2 ± 0.47^b	42.7 ± 0.51^b	37.3 ± 0.88^c	37.6 ± 0.57^c	55.6 ± 0.51^a	57.9 ± 0.92^a	4.78**
	Outgo (% of intake)	82.0 ± 1.94	82.1 ± 0.65	79.0 ± 1.85	79.6 ± 1.96	82.4 ± 1.44	82.0 ± 2.95	
	Apparent absorption (%)	18.0 ± 1.23^b	17.9 ± 0.91^b	21.0 ± 0.71^a	20.4 ± 0.51^a	17.6 ± 0.43^b	18.0 ± 0.68^b	1.48**
Zn	Dietary intake ppm	44.5 ± 0.27^b	44.2 ± 0.28^b	39.1 ± 0.14^c	39.3 ± 0.18^c	57.7 ± 0.24^a	57.9 ± 0.23^a	3.9**
	Outgo (% of intake)	64.6 ± 2.08	64.7 ± 2.39	60.1 ± 2.19	61.2 ± 1.44	64.3 ± 2.04	64.9 ± 2.39	
	Apparent absorption (%)	35.4 ± 2.48^b	35.3 ± 1.60^b	39.9 ± 1.26^a	38.8 ± 1.38^a	35.7 ± 2.18^b	35.1 ± 1.24^b	2.90**
Fe	Dietary intake ppm	244.2 ± 1.12	244.1 ± 1.09	242.7 ± 1.89	241.3 ± 0.28	241.9 ± 0.26	241.6 ± 0.13	NS
	Outgo (% of intake)	74.3 ± 7.47	71.3 ± 7.47	76.1 ± 7.46	75.3 ± 12.8	77.9 ± 3.23	76.1 ± 15.2	
	Apparent absorption (%)	25.7 ± 1.30	28.7 ± 1.26	23.9 ± 0.80	24.7 ± 2.47	22.1 ± 0.85	23.9 ± 2.51	NS

Different superscripts within row differ significantly (** $P < 0.01$).

slightly higher in T_2 (4.5%) and T_3 (2.5%), in T_1 the animals of L-2 consumed about 0.9% more Ca than L-3. Identical differences were observed with P also but in neither of the cases the differences could reach the level of statistical significance.

Trace elements

Intake of Cu, Mn and Zn varied ($P < 0.05$) due to withdrawal and supplementation of these trace elements in the diet (Table 2). Withdrawal of Cu from diet resulted in the lowest intake of Cu in T_2 (10.1 ± 0.02 and 10.0 ± 0.02 ppm, respectively, in L-2 and L-3) though it was optimum as per the NRC (1989) standard (10 ppm). In T_1 Cu intake was higher by almost 19% (11.9 ± 0.05 and 11.8 ± 0.08 ppm in L-2 and L-3 respectively) and the same increased by 38% (13.8 ± 0.01 and 13.8 ± 0.03 ppm in L-2 and L-3 respectively) in T_3. Likewise, animals of L-2 and L-3 consumed, respectively, 8.0% and 6.8% more Mn compared to requirement (40 ppm). Withdrawal of supplementation resulted in decline of Mn intake in both the groups (37.3 ± 0.88 and 37.6 ± 0.57 ppm in L-2 and L-3). Intake increased by 39.2% in T_3 where L-2 and L-3 consumed 55.6 ± 0.51 and 57.9 ± 0.92 ppm Mn respectively. Considering the requirement (40 ppm) Zn intake was 10.9% more in both L-2 (44.5 ± 0.27 ppm) and L-3 (44.2 ± 0.28 ppm) in T_1 and that in T_3 increased by about 44% in both the groups (55.7 ± 0.24 and 57.9 ± 0.23 ppm in L-2 and L-3 respectively). Fe intakes during different treatment periods were much higher than the standard (50 ppm) prescribed by NRC (1989). Paul and Mahapatra (2001) also reported comparable levels of Fe intake in buffalo level while Das *et al.* (2002) observed that high Fe concentration in feeds and fodder might increase the level of dietary Fe in dairy cattle. In the present case also the dietary Fe concentration was fairly high (Table 1) that perhaps resulted in the high dietary Fe intake in all the 3 treatment groups.

Apparent absorption of inorganic nutrients

Ca and P: Apparent absorption of Ca and P from the gut was more dependent on the levels of dietary intake and the interaction between these elements rather than the lactation number of the animals (Table 2). Apparent absorption coefficient (%) of Ca is negatively correlated ($r = -0.51$, $P < 0.05$) with the level of dietary intake. Lowest ($P < 0.01$) apparent absorbability was observed in T_1 (41.1 ± 1.46 in L-2, 41.9 ± 1.48 in L-3) where the maximum Ca intake took place. With little variation in intake absorption also did not differ in T_2 (45.5 ± 0.65 in L-2, 44.9 ± 1.18 in L-3) and T_3 (46.9 ± 0.48 in L-2, 45.7 ± 0.92 in L-3). Absorption of P was more influenced by the level of dietary Ca ($r = -0.69$, $P < 0.01$) than the level of P itself in the diet ($r = -0.38$). The lowest apparent absorption (%) for P was observed in T_1 (53.5 ± 1.82 in L-2, 53.9 ± 2.32 in L-3) when Ca intake was significantly higher. Vitti et al. (2000) and NRC (2000) reported that maximum efficiency of P absorption from the gut took place when the diet contained low to moderate levels of P. The same mechanism operated in this case also as with the decline in P intake apparent absorption increased ($P < 0.01$) in both T_2 (60.8 ± 1.44 in L-2, 60.2 ± 1.44 in L-3) and T3 (59.9 ± 0.84 in L-2, 59.7 ± 1.31 in L-3). Sarkar (2000) and Paul and Mahaptra (2001) also observed that in ruminants a lower dietary level of Ca and P could increase the efficiency of absorption of both these major elements. The present study thus corroborated these earlier reports regarding interaction of inorganic nutrients in the digestive tract.

Trace elements

Absorption of Cu, Mn and Zn was influenced both by the dietary levels of the respective trace elements as well as their interaction in the gut (Table 2). Dietary intake of Cu, Mn and Zn was minimum during T_2 and this facilitated maximizing the efficiency of absorption of these elements in this period of the experiment. The respective absorption coefficients (%) of Cu, Mn and Zn were 18.7 ± 0.61, 21.0 ± 0.71, 39.9 ± 1.26 in L-2 and 18.2 ± 1.08, 20.4 ± 0.51, 38.8 ± 1.38 in L-3. As the intakes increased in T_1 and further in T_3 the amounts of the concerned trace elements going out through feces increased resulting in lower ($P < 0.01$) efficiency of absorption in the corresponding periods. This can further be confirmed by the significantly ($P < 0.05$) negative correlation that was observed between intake and absorption of Cu ($r = -0.51$) and Zn ($r = -0.52$). Identical observations have been reported earlier by Paul and Mahapatra (2001). However, the absorption pattern of these trace elements were almost same in both L-2 and L-3 indicating little influence of lactation number on absorbability of trace elements. The study therefore corroborates the findings of Sarkar (2000) who also failed to observe any difference in the apparent absorption of trace elements in first and second lactating dairy cows. It appears further that the interaction between Cu and Zn influenced the efficiency of absorption of each other. A negative correlation between Zn intake and Cu absorption ($r = -0.56$, $P < 0.01$) was observed in the present experiment. It has been reported that an excess of dietary Zn hindered Cu absorption (Davies and Williams 1977, Oestreicher and Cousins 1982) perhaps by reducing the availability of the latter through formation of metallothionin that eventually gets lost through feces following desquamation of intestinal mucosa. In the present experiment increased dietary level of Zn in T_1 and T_3 resulted in higher fecal loss of Cu and consequently low Cu absorption in the corresponding periods (13.6 ± 0.96 in L-2 and 13.1 ± 1.97 in L-3; 14.9 ± 0.65 in L-2 and 14.6 ± 1.05 in L-3 respectively). Apparent absorption (%) of Zn, though not influenced by dietary Cu level *per se*, was higher than the figures suggested by Hansrad *et al.* (1968) and ARC (1980). However, the present observations (35.4 ± 2.00, 39.4 ± 1.31 and 35.3 ± 1.75 in T_1, T_2 and T_3 respectively) were lower than that observed by Paul and Mahapatra (2001). However, Zn absorption from the digestive tract may range from 10 –

80% (Lall *et al.* 1992, McDowell *et al.* 1992, Lall *et al.* 1999) depending upon the metabolic needs of the animals and other dietary factors (NRC 2000). The present study therefore paves the way for further research on various aspects of intestinal absorption of Zn in ruminants. Efficiency of Fe absorption from the gut was not influenced either by the treatments or by the lactation number. The absorption coefficients ranged from 22.1 ± 2.47% to 25.7 ± 1.30% that corroborate the observations of NRC (1989), Lall *et al.* (1999) and Paul and Mahapatra (2001).

Plasma and milk mineral profile

Neither lactation number nor an increased dietary level of Cu, Mn and Zn influenced plasma concentration of major (Ca and P) elements (Table 3). Mean plasma Ca (mg d l^{-1}) irrespective of lactation number varied between 9.06 ± 0.29 in T_1 to 9.62 ± 0.42 in T_3 and that of P changed from 4.59 ± 0.03 in T_1 to 4.69 ± 0.03 in T_3. The corresponding values in T_2 remained in between (9.00 ± 0.27 and 4.68 ± 0.02 for Ca and P respectively). The Ca and P concentrations found in the present study are well above minimum critical level 8.0 mg dl^{-1} for Ca and 4.5 mg dl^{-1} for P) suggested by Underwood (1977). The present figures corroborate the findings of Haque and Verma (1990) though Ghosh *et al.* (2001b) observed somewhat lower values for both Ca and P in lactating cross bred cows. It is important to note that despite significant decline in intake of Ca and P, only a little change in the plasma concentrations occurred in T_2 and T_3. Perhaps the enhanced efficiency of absorption in the corresponding period coupled with the homeostatic mechanism prevented any significant change in plasma concentrations of these elements. Saba *et al.* (1994) and Cardose *et al.* (1997) also failed to elicit any change in plasma concentrations of Ca and P by altering their level of dietary intake. On the other hand Ghosh *et al.* (2001b) could not find any effect of lactation on identical parameters. The present investigation, therefore, has confirmed these reports made earlier.

Trace element concentrations in plasma followed the trend identical to that observed in case of Ca and P. The plasma concentrations (μg ml^{-1}) of Cu (1.14 ± 0.02 in L-2, 1.18 ± 0.03 in L-3), Fe (1.41 ± 0.03 in L-2, 1.40 ± 0.01 in L-3), Mn (0.43 ± 0.01 in L-2, 0.37 ± 0.02 in L-3) and Zn (1.03 ± 0.01 in L-2, 1.13 ± 0.03 in L-3) irrespective of treatments varied marginally (Table 3) between lactation, though the differences did not reach the level of statistical significance as has been reported earlier also by Ghosh *et al.* (2001b). Levels of dietary intake also could not exert any effect on plasma concentrations of Cu, Mn and Zn. Though the apparent absorption was maximum in T_2 that was not reflected in the plasma (correlation coefficients between apparent absorption and plasma concentrations were 0.21, 0.31 and – 0.08 for Cu, Mn and Zn respectively). Bagley (1997) reported that plasma is not an ideal indicator of dietary Cu status because the compensatory release or uptake of Cu by liver as a homeostatic tool offsets any significant change in plasma concentrations. On the other hand Thomas (1970) and Olson *et al.* (1999) reported that even with a 200 fold increase in dietary Mn level only a negligible change in plasma concentration could be observed. Though such reports are not available with Zn. Lall *et al.* (1994) and Ghosh *et al.* (2001b) failed to exert any effect of dietary Zn level on plasma Zn profile and that obviously support the observations of the present investigation.

Ca and P concentrations of milk did not vary due either to treatment or to lactation number (Table 3). Milk Ca and P concentrations found in the present experiment corroborate the reports of Yadava *et al.* (1998). Ghosh *et al.* (2002b) also did not find any effect of mineral supplementation on major element concentration of milk and the present findings have corroborated these earlier reports in this regard. Trace element

Table 3. Major (Ca and P) and trace (Cu, Fe, Mn and Zn) element profile of plasma and milk

	T_1 (60 days)		T_2 (60 days)		T_3 (60 days)	
	L-2	L-3	L-2	L-3	L-2	L-3
Plasma profile						
Ca mg dl^{-1}	9.25 ± 0.25	8.87 ± 0.29	9.00 ± 0.41	9.10 ± 0.27	9.50 ± 0.64	9.75 ± 0.63
P mg dl^{-1}	4.60 ± 0.04	4.57 ± 0.05	4.65 ± 0.03	4.70 ± 0.03	4.70 ± 0.04	4.67 ± 0.06
Cu μg ml^{-1}	1.11 ± 0.07	1.24 ± 0.25	1.14 ± 0.07	1.17 ± 0.12	1.18 ± 0.08	1.14 ± 0.12
Fe μg ml^{-1}	1.42 ± 0.01	1.42 ± 0.01	1.41 ± 0.01	1.39 ± 0.01	1.41 ± 0.02	1.39 ± 0.02
Mn μg ml^{-1}	0.41 ± 0.05	0.37 ± 0.04	0.45 ± 0.06	0.37 ± 0.03	0.44 ± 0.05	0.37 ± 0.02
Zn μg ml^{-1}	1.04 ± 0.08	1.12 ± 0.12	1.04 ± 0.07	1.13 ± 0.13	1.00 ± 0.04	1.12 ± 0.12
Milk profile						
Ca mg dl^{-1}	0.15 ± 0.03	0.16 ± 0.06	0.15 ± 0.04	0.15 ± 0.04	0.16 ± 0.04	0.16 ± 0.03
P mg dl^{-1}	0.09 ± 0.004	0.09 ± 0.005	0.09 ± 0.005	0.09 ± 0.003	0.09 ± 0.003	0.09 ± 0.002
Cu μg ml^{-1}	0.87 ± 0.07	0.81 ± 0.012	0.81 ± 0.06	0.81 ± 0.06	0.80 ± 0.06	0.75 ± 0.10
Fe μg ml^{-1}	4.05 ± 0.09	3.97 ± 0.19	4.19 ± 0.37	4.19 ± 0.45	4.12 ± 0.30	4.12 ± 0.33
Mn μg ml^{-1}	1.60 ± 0.22	1.60 ± 0.18	1.69 ± 0.06	1.62 ± 0.12	1.69 ± 0.06	1.68 ± 0.06
Zn μg ml^{-1}	3.47 ± 0.21	3.22 ± 0.11	3.56 ± 0.16	3.50 ± 0.10	3.62 ± 0.07	3.50 ± 0.10

concentrations of milk were not affected by mineral supplementation or by lactation number. Vijchulata *et al.* (1994) also could not find any effect of mineral supplementation on milk mineral profile despite an increase in the concentrations of respective mineral elements in blood. Milk Cu was somewhat higher (0.84 ± 0.07, 0.81 ± 0.04 and 0.78 ± 0.06 μg ml^{-1} in T_1, T_2 and T_3 respectively) than the range of 0.37 to 0.43 (Rojas *et al.* 1993). Yadava *et al.* (1998), however, reported that in cow milk Cu concentration may range from 0.01 to 1.15 μg ml^{-1} and moreover, there are reports that supplementation might increase secretion of Cu in milk (Chen *et al.* 1991). Considering the above it can be said that milk Cu concentrations found in the present experiment were within the normal range of variation. Fe concentrations in milk found in the present experiment were 4.01 ± 0.10, 4.19 ± 0.27 and 4.12 ± 0.21 μg ml^{-1} in T_1, T_2 and T_3 respectively. However, Yadava *et al.* (1998) reported that Fe concentration in milk may range from 0.63 – 4.40 μg ml^{-1} and this variation may be related to the contamination of milk with various Fe sources during normal handling of milk (Underwood 1977).

It was concluded from the present study that dietary supplementation of trace elements at different dose levels significantly affects their efficiency of utilization in lactating cows. Maximum efficiency of absorption took place when the diet was either marginally, deficient or just supplied the required quantity. Apparent absorption decreased as the dietary intake increased. However, not much difference could be found between the 20% and 40% levels of increment. Plasma and milk minerals profiles were not influenced nor the lactation number exerted any effect. The study boisters the importance of optimizing the dietary levels of trace elements to maximize the utilization of both organic and inorganic nutrients.

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Relationship between trace mineral content of soil, fodder and animals of different ages and stages of lactation

AJAY KUMAR[1], C SINGH[2] and A P SINGH[3]

Rajendra Agricultural University, Patna, Bihar 800 014 India

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ABSTRACT

An experiment was conducted to estimate the trace minerals in soil, fodder and in blood plasma of animals fed the fodders having known concentration of trace minerals with a view to study the relation between trace mineral content of soil and fodder with trace minerals contents in blood plasma of the crossbred heifers and cows. Twelve different samples of fodder (Sorghum) and soil (from root zone of fodder sample) as well as blood plasma of 6 non-cycling crossbred ((Friesian × Hariana) heifers of each age group of 10 to 13 months, 18 to 21 months and 27 to 30 months, cycling heifers and cycling lactating cows were analysed by atomic absorption spectrophotometer. The concentration of Fe, Cu, Co, Zn and Mn in soil and Sorghum were highly correlated. The concentration of Fe, Cu, Zn, Co and Mn in cycling heifers and lactating cycling cows were significantly ($P<0.01$) higher than the non-cycling heifers of either age group. The concentration of these minerals were at increasing trend as per advancing age of non-cycling heifers. The observation suggests that the trace mineral content of soil affect the fodder content of these minerals. The maintenance of trace mineral in circulation at different ages and reproductive status is also controlled by the intrinsic factor rather than the content of trace minerals in fodders.

Key words: Animal, Fodder, Soil, Trace mineral

Expression of productive potential of dairy cattle largely depends upon the nutrition and management provided to the animals from birth to the whole reproductive life. The role of trace minerals for maintenance of reproductive potential of dairy animals have been well documented (Sharma *et al.* 1988, Desai *et al.* 1982, Qureshi *et al.* 2000). The presence of minerals and trace minerals in the fodders depends on their level in the soil on which the fodders are grown (Singh 1985). With the modern innovation in agricultural technology and use of synthetic fertilizer in large quantities for high yield of grains, it is supposed that the deficiency of trace elements would crop up affecting both plants and animals (Viets 1966, Manickam *et al.* 1977). The concentration of trace minerals in the circulation of the animals in influenced by its content in feeds and fodders fed to the animals (Petukhova and Belov 1992). Keeping in view the relation of trace minerals content of soil, plant and animals the present investigation was undertaken to study the trace mineral content of soil of fodder growing areas of animal production research institute (APRI), Pusa, Bihar; in fodder grown on that soil and in the blood plasma of the crossbred heifers of different age groups and reproductive status fed the fodder having known concentration of trace minerals.

Present address: [1]Junior Assistant Research Officer, Animal Health and Production Institute Patna 14.

[2]Associate Professor and Head, Veterinary Physiology, [3]Associate Professor, Department of Soil Science.

MATERIALS AND METHODS

Collection of soil samples and their processing for trace minerals estimation

Twelve soil samples from root zone of sorghum plant from 12 different site of fodder growing land of APRI, Pusa were collected. Samples were air dried and powdered with the help of wooden plank and roller to make them homogeneous. The trace minerals viz. iron (Fe), copper (Cu), cobalt (Co), zinc (Zn) and manganese (Mn) from processed soil were extracted by Diethyl triamine penta acetic acid (DTPA) (Lindsay and Norvell 1978). Trace elements were estimated by Perkin Elmer Atomic Absorption Spectrophotometer (Model A Analyst 100).

Collection of fodder samples and their processing for trace mineral estimation

Twelve fodder fields were selected in a plot from where green fodders (sorghum) were being fed to the experimental animals. Sorghum plants from all those 12 fields were collected, chopped and washed in acidified detergent solution

(about N/10 HCl + a small portion of detergent). The detergent washed fodder samples were subsequently washed by distilled water, dried in air followed by hot air circulation in over at oven at 65° C and pulverised in waring blender to make it homogeneous. The ashing of the samples was done according to Sakal *et al.* (1984). The concentration of Fe, Cu, Co, Zn and Mn were estimated in the fodder sample by Perkin Elmer Atomic Absorption Spectrophotometer.

Experimental animals and feeding schedule

Six noncycling crossbred heifers of each age group of 10 to 13 months, 18 to 21 months and 27 to 30 months, 6 cycling crossbred (Friesian 87.50% × Hariana 12.5%) heifers of 30 to 36 months and 6 cycling lactating crossbred (Friesian 75% × Hariana 25.0%) cows (3rd to 4th lactation) of 5 to 7.5 years of age were selected from animals herd of APRI. Animals were fed Sorghum (green fodder) *ad lib.* during morning (6 to 7AM) and evening (4 to 5PM). The lactating cows were provided additional production ration consisting of green (Sorghum) fodder supplemented with concentrate @ 1 kg/ 3kg of milk over 3 kg daily milk production as practised in the farm. All the animals were being kept loose without chaining but confined in fence area having cattle shed to allow them for free movement. They were provided fresh drinking water throughout the day and night. This feeding and managements were provided continuous for 30 days.

Blood samples collection and analysis of trace minerals

On day 30 after feeding experimental green fodder (Sorghum) blood samples (20 ml) were collected by jugular veinipuncture from each animal during morning (4 to 5 AM) hours in chilled heparinized glass test tubes using sterilized disposable needle (18 gauge) and syringe (20 ml). Plasma was separated within 2 hr of blood collection by centrifuging at 300 rpm for 20 min. Plasma separated were divided in 5 different aliquots and preserved in deep freeze at –20° C. The trace minerals were estimated in plasma by Atomic Absorption Spectrophotometer within 24 to 48 hr after blood sample collection by the methods as described by Saxena and Gupta (1993).

The data were analysed for standard error averages and 2 way analysis of variance to elucidate the differences among the individual groups. The correlation of different trace minerals concentration between soil and plant were also calculated (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The trace minerals content in soil and fodder (sorghum) have been presented in Table 1 while in circulating plasma of animals have been presented in Table 2. The content of Iron (Fe), copper (Cu), cobalt (Co) and manganese (Mn) in both soil and fodder collected from 12 different sites varied. The Fe, Cu, Co, Zn and Mn content in soil and fodder samples were highly correlated.

The concentration of Fe, Cu, Co, Zn and Mn detected in circulating plasma of 10 to 10 months heifers, were lower than 18 to 21 and 27 to 30 months noncycling heifers whereas Fe and Co concentration in 10 to 13 months and 18 to 21 months old heifers were significantly ($P<0.01$) lower than the noncycling heifers of 27 to 30 months. Magnitude of Fe, Co, Zn and Mn in noncycling heifers of all age groups and Cu in 10 to 13 months and 18-21 months heifers were significantly ($P<0.01$) lower than cycling heifers and cycling lactating cows. However, the plasma concentration of Fe, Cu, Co, Zn and Mn of cycling heifers and cycling lactating cows were at par (Table 2).

The Fe and Cu content of the soils were within the range of Fe and Cu content of the best fodder growing lands of Bihar (Sakal *et al.* 1996). None of the soil samples had lower

Table 1. Trace mineral concentration (ppm) in soil of fodder growing area and in fodder (Sorghum) fed to the animals

No. of soil	Trace minerals									
and fodder	Fe		Cu		Co		Zu		Mn	
samples	Soil	Fodder	Soil	Fodder	Soil	Fodder	Soil	Fodder	Soil	Fodder
S.F.1	35.43	523.7	2.06	6.8	0.26	30.	0.66	21.8	26.97	40.1
S.F.2	34.48	515.6	1.94	6.3	0.28	3.2	0.81	24.3	27.10	42.3
S.F.3	11.73	207.1	0.80	3.5	0.48	6.9	0.40	16.7	10.95	19.9
S.F.4	12.2	267.7	3.49	7.3	0.39	4.8	0.16	10.6	14.78	34.5
S.F.5	30.1	502.0	1.71	6.1	0.38	4.0	1.00	25.5	23.59	38.8
S.F. 6	30.47	513.5	2.19	6.9	0.49	7.2	1.83	61.2	22.30	37.0
S.F.7	18.51	340.0	1.14	5.5	0.38	4.8	0.55	20.4	12.10	29.5
S.F.8	18.75	384.7	1.21	3.7	0.39	4.8	0.53	17.8	10.88	17.5
S.F.9	16.24	315.5	1.03	4.4	0.45	6.1	0.59	20.8	15,27	36.0
S.F.10	19.69	448.5	1.04	5.1	0.34	°3.4	0.75	22.0	11.00	28 7
S.F.11	21.32	486.3	1.40	6.1	0.21	2.8	1.26	49.3	13.45	29.6
S.F.12	18.97	417.8	1.28	5.9	0.42	5.5	1.10	31.9	15.28	36.1
Mean	22.32±	410.2±	1.61±	5.8±	0.37±	4.71±	0.80±	26.86±	16.98±	32.5±
± SE	2.37	30.96	0.21	0.31	0.03	0.34	0.13	4.17	1.80	2.23

Table 2. Trace mineral concentration (ppm) in animals

Particular of samples	Fe	Cu	Co	Zn	Mn
10 to 30 months heifers (6)	2.70±20.14[a]	1.03+0.09[a]	0.27+0.01[a]	1.22+0.06[a]	0.30+0.02[a]
18 to 21 months heifers (6)	2.90+0.06[a]	1.08+0.07[a]	0.32+0.01[a]	1.32+0.06[a]	0.32+0.01[a]
27 to 30 months heifers (6)	3.12+0.07[b]	1.18+0.07[a]	0.43+0.2[b]	1.40+0.11[a]	0.35+0.01[a]
Cycling heifers (6)	4.41+0.13[d]	1.36+0.03[b]	0.97+0.04[c]	2.16+0.05[b]	1.01+0.01[b]
Cycling lactating cows (6)	4.10+0.10[d]	1.41+0.08[b]	1.13+0.04[c]	2.28+0.07[b]	0.99+0.03[b]

NB: (i) The figures bearing common superscripts (column wise) do not differ significantly, (ii) figures in parenthesis show the number of animals.

than the critical levels of Fe, Cu, Co and Mn. Minimum critical level of Fe and Cu in soil have been reported to be 6.95 and 0.66 ppm respectively (Sakal *et al.* 1984). The Co content of soil was also within the range required for the best fodder growing area (Singh, 1985).

However, out of 12 soil samples Zn content of 60% samples were below the critical level (0.78 ppm; Sakal *et al.* 1982). Similarly 67% of fodder samples were deficient in Zn content.

Manganese content of soil in present investigation was 4 folds than the critical level of Mn reported (Sakal *et al.* 1990). However, the mean value of Mn in soil was within the range in fodder growing areas of Bihar (Sakal *et al.* 1996). The existence of high correlation between all trace mineral elements of soils and plants during present investigation confirm the previous reports (Viets 1966 Manickam *et al.* 1977).

The circulating plasma Fe, Cu, Co, Zn and Mn concentration in noncycling heifers of present experiment were comparable to the plasma Fe and Cu (Amrith *et al.* 1973), Co (Petukhova Khasan 1981), Zn Swenson and Reece (1996) and Mn (Parmar *et al.* 1986) concentration reported in cows and heifers. Gradual increase of all trace minerals in circulating plasma of noncycling heifers as per advancing age were supported by a number of worker; higher Fe concentration in cattle (Prasad and Rao 1997) and buffaloes (Nayyer *et al.* 1996), Cu concentration in cattle (Desai *et al.* 1982), Co in cattle (Valyushkin 1974), Zn in buffalo heifers (Dabas *et al.* 1997) and Mn concentration in cattle (Tambe *et al.* 1998).

Significantly higher concentration of all trace minerals in cycling heifers and cycling lactating cows than noncycling heifers at same feeding and managemental condition suggests that either hormonal factors or the trace minerals itself is responsible to activate the enzymes and endocrine systems of the pubertal and sexually matured animals to trigger the activity of hypothalmohypophyseal and gonadal system to bring the animal cyclicity. Trace minerals might have caused the elevation of FSH and FSH: LH ratio resulting into folliculogenesis and resumption of estrous cyclicity. Significantly higher concentration of Fe and Cu (Sharma *et al.* 1988), Zn (Prasad and Rao 1997), Co (Valyushkin 1982) and Mn (Wilson 1966), in cycling than noncycling animals have been reported.

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Mineral distribution in soil, feeds and grazing cattle of different physiological stages in the red laterite and new alluvial agroclimatic zones of West Bengal

A DAS[1], T K GHOSH[2] and S HALDAR[3]

West Bengal University of Animal and Fishery Sciences, 37, K. B. Sarani, Kolkata, West Bengal 700 037 India

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ABSTRACT

A survey was carried out in the red laterite and new alluvial zone of West Bengal to compare the status of major (Ca and P) and trace (Fe, Cu, Mn and Zn) elements in cattle population grazing over 2 different regions viz. red laterite and new alluvial agroclimatic zones. Concentrations of Fe, Cu, Mn and Zn in soil were above the minimum critical levels in both the agroclimatic zones. However, 100% of the samples of paddy straw were deficient in Ca, P and Mn in both the regions. Cu deficiency was also widespread in various concentrate feeds, paddy straw and green roughage. The highest plasma concentration of Ca and P was observed in the calves in either of the zones. On the other hand, lowest concentrations of Fe, Cu and Mn in plasma were observed in calves that increased with an advancement of age in both the zones. Mn in calves was lower than the minimum critical levels in either zone though other minerals did not show any deficiency. Plasma Fe and Cu were higher ($P < 0.05$) in the animals of red laterite zone perhaps due to the richness of soil, feeds and fodder of red laterite zone in these mineral elements. The reverse was the case for Zn, the concentration of which was higher in the soil, feeds and fodder of new alluvial zone. Liver Cu was below the minimum critical concentration in either of the zones indicating deficiency of Cu in the grazing livestock. The study emphasises on strategic supplementation of specific mineral elements to augment the productivity of grazing cattle.

Key words: Feeds and fodder, Grazing cattle, Mineral elements, New alluvial zone, Physiological stage, Red laterite zone, Soil

Assessment of mineral status in grazing ruminants has been considered to be an important criteria to increase animal productivity especially in the tropics and subtropics where imbalances or deficiencies of various major and trace elements in animal feeds and fodder are very common (McDowell *et al.* 1984). In West Bengal livestock are maintained mainly on grazing with little access to any mineral supplement (Das *et al.* 2002). However, deficiency of major and trace elements in tropical feeds and fodder is a widespread problem (McDowell *et al.* 1984, Tejada *et al.* 1987) which often limits the performance of grazing ruminants. Mineral concentrations in blood provide an indication regarding the status of mineral intake in the grazing ruminants notwithstanding the interactions taking place between different major and trace elements present in the soil, plants as well as in the animal system itself. Furthermore, ascertaining the concentrations of the same in various vital organs can also be of use to detect the actual distribution of the same in body (Underwood 1981). The present investigation was carried out in 2 different agroclimatic zones of West Bengal to ascertain how the pattern of distribution of various major and trace elements differs in the soil feeds, fodder,as well as in animal system with a change in soil character and other agroecological parameters.

Present address: [1]M.V.Sc. Scholar, [2]Professor, [3]Lecturer, Department of Animal Nutrition.

MATERIALS AND METHODS

The investigation was carried out in 2 agroclimatic regions viz. red laterite (zone-1) and new alluvial (zone-2) zones spreading, respectively, over Purulia and Nadia districts of West Bengal. The annual mean temperature of the former is 27°C (7°C to 39°C) and that in the latter is 28°C (10°C to 38°C). The average annual rainfall in the red laterite zone is 146 mm (8 to 285 mm) and the relative humidity ranges between 23 to 99%. In the new alluvial zone, on the other hand, the average annual rainfall is 130.5 mm (5 to 270 mm) and relative humidity varies from 55 to 93%. In the red laterite zone the soil pH ranges from 6.6 to 7.2 while in the new alluvial zone, where the soil is clay loam in type, pH varies widely from 6.5 to 8.9.

Categorization of animals

The animal population under survey consisted of crossbred animals of different age groups. These animals were divided

into 5 categories according to age and physiological parameters viz. calves (upto 1 year of age), heifers (from 1 year up to first calving), lactating animals, dry (pregnant) animals and bullocks.

Collection of samples

Samples of soil, feeds, fodder, animal blood and tissue were collected from both the agroclimatic zones. About 250g of soil samples were collected at plough depth (20cm to 25cm) from different areas of pasture and cultivable land of the respective zones and were stored in plastic packets. The packets were prewashed with triple distilled water and air-dried to prevent any contamination. Approximately 250g of different concentrate feed ingredients consumed by the cattle population of the red laterite and new alluvial zones were collected randomly from the farmers. Randomly collected samples of pasture grass, as well as paddy straw were dried and kept for further analysis of major and trace elements. Blood samples were collected by jugular venupuncture from 100 cattle (50 from each zone) of different age groups. Plasma separated by centrifuging the samples at 3 000 rpm for 20 min were stored at –20°C for subsequent analysis of major and trace elements. About 10g of liver, kidney and lung tissues were collected from the local slaughterhouses of the respective zones. 12 samples (6 from each zone) for each of the above mentioned vital organs were collected which after processing (Fick *et al.* 1979) were analysed for Fe, Zn, Cu and Mn.

Analytical techniques

Soil samples (10g) were extracted with 0.005 M diethylene triamine pentaacetic acid (DTPA) solution for 2 hr at pH 7.3 (Lindsay and Norvell 1978). Following filtration the filtrate was analyzed for Fe, Zn, Cu and Mn with atomic absorption spectrophotometer (Perkin Elmer A Analyst 100). Feeds and fodder samples (0.25g) were extracted with triacid mixture (containing concentrated HNO_3, H_2SO_4 and $HClO_4$, 6: 4: 1 v / v) at 180°C (AOAC 1980) for estimation of major (Ca and P) and trace (Fe, Zn, Cu and Mn) elements by atomic absorption spectrophotometry. Plasma (1 ml) and dry samples (1g) of liver, kidney and lung were extracted with triacid mixture of HNO_3, H_2SO_4 and $HClO_4$ (9: 2: 1 v / v) at 180 – 200°C for estimation of Ca, P, Fe, Zn, Cu and Mn by atomic absorption spectrophotometer.

Statistical analysis

The data was analyzed (Snedecor and Cochran 1967) using MS excel software package. 't'-test was performed to find out the differences in trace element concentrations of soil in different agroclimatic zones as well as the feeds and fodder of the respective zones. The plasma and tissue concentrations of various major and trace elements observed in different categories of animals were subjected to ANOVA to ascertain the differences between the categories and agroclimatic zones.

RESULTS AND DISCUSSION

Trace elements in soil

Concentrations of trace elements (Fe, Cu, Mn and Zn) in both the red laterite (zone-1) and new alluvial (zone-2) agroclimatic zones were above the respective minimum critical levels (Table 1). Higher concentrations of Fe ($P < 0.01$) and Cu ($P < 0.05$) were observed in zone-1 while in zone-2

Table 1. Profile of trace elements of soil

Trace element	Critical level#	Zone-1 (n = 5)	Zone-2 (n = 5)	Significance
pH	-	6.6-7.2	6.5-8.9	
Fe ppm	<2.5	107.7 ± 4.19^a (97.5 – 117.3)	56.7 ± 2.89^b (50.3 – 62.8)	$P < 0.01$
Cu ppm	< 0.3	2.2 ± 0.20^c (1.9 – 2.7)	1.5 ± 0.04^d (1.4 – 1.6)	$P < 0.05$
Mn ppm	< 5.0	41.3 ± 3.24^e (33.3 – 47.6)	95.0 ± 5.60^f (83.1 – 107.4)	$P < 0.01$
Zn ppm	< 1.0	1.2 ± 0.29^g (0.8 – 1.1)	2.0 ± 0.16^h (1.7 – 2.4)	$P < 0.05$

#Critical level of soil trace element concentration (Viets and Lindsay 1973). Figures in parenthesis indicate ranges of respective trace elements. Different superscripts within row indicate significant variation.

Mn ($P < 0.01$) and Zn ($P < 0.05$) concentrations were on higher side. None of the soil samples was deficient in these trace elements. Fe in both zone-1 (107.7 ± 4.19 ppm) and zone-2 (56.7 ± 2.89 ppm) was much higher than the minimum critical level (< 2.5 ppm) and this appears to be a characteristic feature of this agroclimatic zone (Das *et al.* 1990, Das *et al.* 1993). Soil Mn varied widely between the 2 agroclimatic zones (41.3 ± 3.24 ppm in zone-1, 95.0 ± 5.60 ppm in Zone-2) which may be related to soil pH. Mn solubility is very much sensitive to change in soil pH (Rojas *et al.* 1993). In zone-1 soil pH was within a range of 6.6 to 7.2 while that in Zone-2 varied widely from 6.5 to 8.9 which might have increased the solubility and hence the amount of extractable Mn. Chauhan and Nderingo (1997) reported identical Mn concentrations in tropical soil while Das *et al.* (1993) reported that Mn in new alluvial and red laterite soil of West Bengal may vary widely from 13.8 to 109.3 ppm. Cu in new alluvial soil (1.5 ± 0.04 ppm) was comparatively lower than that in the red laterite soil (2.2 ± 0.20 ppm) and the findings therefore corroborate the report of Sarkar *et al.* (1992) who observed lower concentration of soil Cu in the new alluvial agroclimatic zone.

Feeding practices

Since the study was concerned with only grazing cattle, therefore the sampling was confined to those farmers who kept their animals mainly on grazing. The pasture consisted of the locally grown grasses among which *doob* grass (*Cynodon dactylon*) was ubiquitous in both zone1 and 2. In

absence of any other supplementary green fodder this grass was supposed to supply the main bulk of green roughage. However, in zone-1 *Paspalum conjugatum*, *Tephrosia purpurea* and *Bluneria lacera* were the other species of green roughage which were also being consumed by the animals in certain areas. Similarly in zone-2 *Karma* (*Ziziphus rotundifolia*) and *mutha* (*Eupatorium odoratum*) grasses were the other most common types of green roughage consumed by the animals along with *doob* grass. In addition the animals received paddy straw (4 to 6 kg day^{-1}) as bulk roughage supplement. In both the agroclimatic zones the farmers supplied their animals with mustard-cake or deoiled rice bran either singly or in combination with maize grain and gram *chuni*. The amount of concentrate supplement, however, varied with the economic status of the farmer. Interestingly amongst the entire surveyed population not even a single family was found to supplement their animals with any mineral source.

Mineral status of feeds and fodder

Concentrations of major (Ca and P) and trace (Fe, Zn, Cu and Mn) elements in different concentrate ingredients, paddy straw, pasture grass and other green roughage as related to different agroclimatic zones are presented in Table 2. Ca in the concentrate feeds of zone-1 (0.8 ± 0.18%) was higher ($P < 0.05$) than that found in identical ingredients of zone-2 (0.6 ± 0.14%). Based on a minimum critical level of 0.3%, 30.3% of concentrate feed samples were deficient in zone-2 while in zone-1 the corresponding figure was 14.8%. Unlike Ca, P was adequate in the concentrate feeds of zone-1 (1.1 ± 0.16%) with none of the samples indicating deficiency. In zone-2, however, 18.2% of the samples exhibited P deficiency (mainly gram chuni, having P content ranging from 0.16 to 0.21%) based on the minimum critical level of 0.25%. All samples of paddy straw were deficient in Ca and P in both zones1 and 2 which corroborate the characteristic feature of tropical dry forage (McDowell 1984, Prabowo *et al.* 1990, Gowda *et al.* 2002). Among the pasture grass and green roughage deficiency of P was more severe in zone-1 than in zone-2 ($P < 0.01$). Morillo *et al.* (1989) reported that most of the tropical grasses including *Paspalum* sp., a common green roughage for the cattle and buffaloes of zone-1, were low in P than the improved varieties of grasses growing under similar condition. Das *et al.* (2002) observed that in the new alluvial zone of West Bengal P in cultivated fodder might be as low as 0.09 to 0.15%. The present findings, hence, have confirmed the earlier reports (Das *et al.* 1993, Sarkar *et al.* 1994, Chauhan and Nderingo 1997) indicating that P deficiency has been one of the most severe mineral limitations to the grazing livestock of these agroclimatic zones of the tropics.

Considering the minimum critical level of 50 ppm none of the feeds and fodder samples was deficient in Fe. The high levels of Fe, which could partly be attributable to the soil and

Table 2. Major and trace element contents of feeds and fodder (on DM basis)[a]

Critical level[#]	Ca% < 0.30	P% < 0.25	Fe ppm < 50.0	Zn ppm < 30.0	Cu ppm < 8.0	Mn ppm < 40.0
Zone-1	0.8 ± 0.18*	1.1 ± 0.16	364.1 ± 47.63	47.9 ± 9.65	26.0 ± 5.37	64.2 ± 12.50
(n = 27)	(0.2 – 2.1)	(0.4 – 1.6)	(143.3 – 593.3)	(4.5 – 96.4)	(6.2 – 63.2)	(14.5 – 105.9)
% Deficient	14.8	–	–	25.9	14.8	29.6
Concentrate feed ingredients[b]						
Zone-2	0.6 ± 0.14*	1.1 ± 0.21	373.0 ± 50.00	48.5 ± 8.99	25.4 ± 3.76	59.8 ± 9.56
(n = 33)	(0.2 – 1.9)	(0.2 – 1.9)	(5.9 – 52.3)	(12.4 – 109.4)	(5.9 – 52.3)	(16.2 – 113.3)
% Deficient	30.3	18.2	–	30.3	12.1	9.1
Paddy Straw						
Zone-1	0.15 ± 0.01	0.04 ± 0.02	262.5 ± 9.92*	32.3 ± 0.56*	10.6 ± 0.31*	22.3 ± 0.27
(n = 9)	(0.13 – 0.18)	(0.03 – 0.04)	(246.5 – 289.4)	(31.2 – 33.3)	(9.7 – 11.0)	(21.6 – 22.9)
% Deficient	100.0	100.0	–	–	33.3	100.0
Zone-2	0.13 ± 0.01	0.06 ± 0.01	224.4 ± 10.05*	41.1 ± 0.30*	8.3 ± 0.52*	23.9 ± 0.41
(n = 8)	(0.10 – 0.15)	(0.04 – 0.07)	(200.3 – 241.6)	(40.4 – 41.9)	(7.0 – 9.3)	(23.2 – 25.1)
% Deficient	100.0	100.0	–	–	50.0	100.0
Pasture grass and green roughage						
Zone-1	0.49 ± 0.07*	0.21 ± 0.02*	323.1 ± 37.18*	22.7 ± 2.41	6.2 ± 0.52*	71.8 ± 4.52
(n = 18)	(0.21 – 0.79)	(0.16 – 0.31)	(130.3 – 450.2)	(9.6 – 29.9)	(3.9 – 8.5)	(57.4 – 98.3)
% Deficient	33.3	72.2	–	100.0	72.2	–
Zone-2	0.42 ± 0.04*	0.26 ± 0.03*	297.8 ± 39.13*	24.9 ± 2.71	4.9 ± 0.51*	78.9 ± 5.44
(n = 14)	(0.26 – 0.59)	(0.16 – 0.35)	(161.5 – 410.3)	(16.5 – 35.2)	(2.5 – 6.1)	(65.2 – 103.3)
% Deficient	14.3	42.9	–	57.1	100.0	–

Critical level of major and trace elements in feeds and fodder (Prabowo *et al.* 1990); *indicates significant difference at $P < 0.05$. a represents means values. Figures in parenthesis indicate ranges of respective mineral elements. b represents mean values of maize grain, mustard oil-cake, gram *chuni*, rice bran.

surface contamination, are in agreement with the findings of Das *et al.* (2002) and Gowda *et al.* (2001, 2002). Fe in feeds and fodder was highly correlated with soil Fe in both zone-1 ($r = 0.92$) and zone-2 ($r = 0.96$) and consequently Fe concentrations in paddy straw and green roughage were lower ($P < 0.05$) in zone-2 (224.4 ± 10.05 ppm in paddy straw, 297.8 ± 39.13 ppm in green roughage) compared to that in zone-1 (262.5 ± 9.92 ppm in paddy straw, 323.1 ± 37.18 ppm in green roughage). Higher levels of Fe in soil and fodder samples of zone-1 might be related to the comparatively lower soil pH of that area which perhaps facilitated the availability and uptake of Fe by the plants as reported earlier by McDowell *et al.* (1984) and Valdes *et al.* (1988).

Zn, Cu and Mn concentrations in various concentrate ingredients did not vary between zone 1 and 2. However, there are variations in individual micronutrient contents in different concentrate feeds. All the samples of maize grains in both the zones were deficient in Cu. 100% samples of maize grain in zone-2 were deficient in Zn and Mn also. Gram *chuni*, one of the most common concentrate ingredients in the new alluvial zone of West Bengal, was deficient in Zn as well as in Mn. Cu in paddy straw as well as in the green roughage was higher ($P < 0.05$) in zone-1 (10.6 ± 0.31 ppm in paddy straw, 6.2 ± 0.52 ppm in green roughage) than that in zone-2 (8.3 ± 0.52 ppm in paddy straw, 4.9 ± 0.51 ppm in green roughage). A highly positive soil- feed correlation was observed in both zone-1 ($r = 0.98$) and zone-2 ($r = 0.97$) which partially explains the lower Cu concentrations in the feeds and fodder of zone-2 where soil Cu was lower ($P < 0.05$) than zone-1. Consequently the extent of Cu deficiency was more in paddy straw and green roughage of zone-2 (50.0% and 100% respectively) vis-a-vis that of zone-1 (33.3% and 72.2% respectively). Mn deficiency was 100% in paddy straw of both zone-1 (21.6 – 22.9 ppm) and zone-2 (23.2 – 25.1 ppm) which is characteristic of tropical forage (Valdes *et al.* 1988, Knebusch *et al.* 1988). Zn deficiency in green roughage was more predominant in zone-1 (100% deficiency) compared to zone-2 (57.1% deficiency) and the present findings corroborate that of Das *et al.* (2002) and Gowda *et al.* (2002). These deficiencies of microelements in the feeds and fodder may partially be explained by the soil pH which was in neutral to alkaline side in both the agroclimatic zones. Such a pH causes a decrease in the relative availability of microelements in plants (McDowell *et al.* 1984) and this perhaps has resulted in the moderate to severe deficiency of Zn, Cu and Mn in the feeds and fodder.

Plasma mineral profile

Plasma concentrations of major (Ca and P) and trace (Fe, Zn, Cu and Mn) elements varied with the agroclimatic zones as well as with the physiological categories of the animals (Table 3).

Plasma, Ca, though did not follow any definite trend, was within the normal range of variation and none of the samples was found deficient (based on minimum critical level of 8.0 mg dl^{-1}). The present observations corroborate the earlier reports (Haldar *et al.* 1995, Das *et al.* 2002) and have also confirmed that in cattle Ca deficiency is not likely to occur under grazing conditions (Underwood 1981). It is noteworthy that the highest plasma Ca concentration was observed in the calves (12.7 ± 0.20 $mgdl^{-1}$ in zone-1, 12.3 ± 0.15 $mgdl^{-1}$ in zone-2) followed by the heifers (11.2 ± 0.30 $mgdl^{-1}$ in zone-1, 10.8 ± 0.30 $mgdl^{-1}$ in zone-2). These higher ($P < 0.05$) concentrations in the animals of lower age group might be due to increased osteoblastic activities taking place in the growing animals during the process of bone development. It further becomes evident that as age of the animals progressed the extent of grazing increased although the deficiency of

Table 3. Plasma concentration of major and trace elements in cattle of different physiological stages*

Major/trace element	Critical level ‡	Agroclimatic zone	Physiological category of cattle					Significance$
			Milch	Dry	Heifer	Bullock	Calf	
Ca $mgdl^{-1}$	< 8.0	Zone-1	9.3 ± 0.26^{a}	10.4 ± 0.15^{c}	11.2 ± 0.30^{d}	9.7 ± 0.20^{b}	12.7 ± 0.20^{e}	$P < 0.05$
		Zone-2	9.9 ± 0.24^{b}	9.4 ± 0.12^{a}	10.8 ± 0.30^{c}	10.0 ± 0.22^{b}	12.3 ± 0.15^{e}	
P $mgdl^{-1}$	< 4.5	Zone-1	4.7 ± 0.17^{b}	4.2 ± 0.10^{a}	5.8 ± 0.12^{cd}	4.9 ± 0.24^{b}	6.7 ± 0.16^{e}	$P < 0.05$
		Zone-2	4.9 ± 0.30^{b}	5.4 ± 0.17^{bd}	5.6 ± 0.17^{cd}	5.1 ± 0.18^{b}	6.8 ± 0.19^{e}	
Fe μgml^{-1}	< 1.00	Zone-1	5.7 ± 0.15^{e}	5.0 ± 0.15^{d}	3.8 ± 0.07^{c}	3.9 ± 0.40^{c}	3.0 ± 0.49^{ab}	$P < 0.05$
		Zone-2	4.0 ± 0.10^{c}	3.7 ± 0.08^{c}	3.1 ± 0.10^{ab}	3.4 ± 0.51^{b}	2.7 ± 0.08^{a}	
Zn μgml^{-1}	< 0.60	Zone-1	1.01 ± 0.03^{a}	1.33 ± 0.11^{b}	1.58 ± 0.08^{c}	1.13 ± 0.08^{a}	1.76 ± 0.20^{d}	$P < 0.05$
		Zone-2	1.39 ± 0.06^{b}	1.50 ± 0.09^{c}	1.85 ± 0.17^{d}	1.48 ± 0.10^{c}	2.10 ± 0.08^{e}	
Cu μgl^{-1}	< 0.65	Zone-1	1.59 ± 0.05^{f}	1.33 ± 0.10^{e}	0.79 ± 0.05^{d}	0.83 ± 0.05^{b}	0.66 ± 0.05^{b}	$P < 0.05$
		Zone-2	0.83 ± 0.04^{d}	0.85 ± 0.02^{d}	0.70 ± 0.05^{c}	0.75 ± 0.03^{cd}	0.53 ± 0.03^{a}	
Mn μgml^{-1}	< 0.20	Zone-1	0.65 ± 0.02^{d}	0.64 ± 0.03^{d}	0.46 ± 0.02^{b}	0.53 ± 0.04^{c}	0.14 ± 0.02^{a}	$P < 0.05$
		Zone-2	0.74 ± 0.03^{e}	0.67 ± 0.02^{d}	0.51 ± 0.03^{c}	0.66 ± 0.05^{d}	0.18 ± 0.03^{a}	

* Observation based on 50 animals (10 from each category) from each zone. Total number of samples 100. ‡ Critical level suggested for cattle (McDowell *et al.* 1984). $ Different superscripts within rows or columns (each mineral to be considered separately) differ significantly.

Ca, that was observed in the concentrate feeds (14.8% and 30.3% deficiency in zones1 and 2 respectively), paddy straw (100% deficiency in both the zones) and green roughage (33.3% and 14.3% deficiency in zones1 and 2 respectively), has not been reflected at all in the plasma perhaps due to extensive mobilization of bone Ca which takes place to maintain the Ca homeostasis under a deficient feeding regime.

Unlike Ca plasma P followed a definite trend and was found to have an inverse relationship with the age of the animals. The highest plasma P was observed in the calves in both the zones (6.7 ± 0.16 mgdl^{-1} in zone-1, 6.8 ± 0.19 mgdl^{-1} in zone-2) followed by the heifers (5.8 ± 0.12 mgdl^{-1} and 5.6 ± 0.17 mgdl^{-1} in zones1 and 2 respectively). P in the plasma of bullocks, dry and milch animals was lower ($P < 0.05$) than that in the calves and heifers. The activity of alkaline phosphates, an enzyme having P as key component, is supposed to be higher in the calves and heifers as osteoblastic activities are likely to be more in the animals of growing phase as has been reported earlier (Haque and Verma 1990, Das *et al.* 2002). Morillo *et al.* (1989) reported that under grazing condition P deficiency is most likely to occur in lactating and pregnant dry animals perhaps due to an increased requirement of P in the process of galactopoiesis and fetal development. However, in the present investigation no such trend was identified and plasma P in the milch animal category was normal in both Zones 1 (4.7 ± 0.17 mgdl^{-1}) and 2 (4.9 ± 0.30 mgdl^{-1}) and this corroborates the report of Das *et al.* (2002). However, in dry animal category, which included pregnant animals, plasma P was deficient (minimum critical level 4.5 mgdl^{-1} suggested by McDowell *et al.* 1984) in almost all the surveyed animals of zone-1 (4.2 ± 0.10 mgdl^{-1}) though in zone-2 the same was in the normal range (5.4 ± 0.17 mgdl^{-1}). Screening of plasma P levels in all categories of animals indicates that animal in zone-1 had marginally lower ($P > 0.05$) P compared to that in zone-2. A lower P profile of feeds and fodder of zone-1 coupled with a higher ($P < 0.05$) Fe contents in the green, as well as dry roughage might be one of the responsible factors. High Fe content in feeds and fodder causes precipitation of inorganic P making it unavailable to the plant species and subsequently to the animals (Prabowo *et al.* 1988, 1990). Pregnancy might have acted as a confounding factor resulting in the lowest plasma P concentration in this category of animals of zone-1.

Plasma Fe was higher ($P < 0.05$) in the animals belonging to zone-1. Perhaps the higher Fe profile of feeds and fodder of zone-1 was reflected in the plasma and this is in agreement with the findings of Das *et al.* (1997) related to the plasma mineral profile of crossbred cattle grazing over identical agroclimatic conditions. None of the animals in either zone was deficient in Fe which has further confirmed the earlier reports of Das *et al.* (1997) and Das *et al.* (2002) who observed that Fe deficiency was least likely to occur in the grazing livestock of red laterite and new alluvial zones owing to the fairly high concentrations of Fe in the soil, feeds and fodder of these regions. It is important to note that plasma Fe was significantly lower ($P < 0.05$) in the calves of both Zones 1 (3.0 ± 0.49 μgml^{-1}) and zone-2 (2.7 ± 0.08 μgml^{-1}). Perhaps consumption of dam's milk, which is a naturally poor source of Fe (McDowell 19992), resulted in such lower plasma levels. Plasma Zn (μgml^{-1}) was lower ($P < 0.05$) in the animals of zone-1 (1.01 ± 0.03, 1.33 ± 0.11, 1.58 ± 0.08, 1.13 ± 0.08 and 1.76 ± 0.20 in milch animals, dry animals, heifers, bullocks and calves respectively) vis-a-vis that in zone-2 (1.39 ± 0.06, 1.50 ± 0.09, 1.85 ± 0.17, 1.48 ± 0.10 and 2.10 ± 0.08 in milch animals, dry animals, heifers, bullocks and calves respectively). Identical was the trend for plasma Mn also though in this case the differences did not reach the level of statistical significance in calves and dry animals. Perhaps the deficiency of Zn coupled with higher Fe concentration in the feeds and fodder of zone-1 affected the utilization of Zn and also that of Mn, as was reported by Prabowo *et al.* (1988, 1990), and this has been reflected in the plasma concentrations. Based on a minimum critical plasma concentration of 0.60 μgml^{-1} (McDowell *et al.* 1984) none of the plasma samples was deficient in Zn despite its moderate to severe deficiency in the feeds and fodder of either zone (Table 2). In both the agroclimatic zones plasma Zn was higher ($P < 0.05$) in the calves and this is in agreement with earlier reports (Sawadogo *et al.* 1988, Van Akess *et al.* 1991, Das *et al.* 2002). The lower plasma Zn in milch cattle might be due to heavy drainage of Zn through milk (McDowell 1992) which in turn resulted in increased plasma Zn levels in calves reared on milk. On the hand higher ($P < 0.05$) plasma Mn was observed in the lactating and dry as well as pregnant animals in both zones 1 (0.65 ± 0.02 and 0.64 ± 0.03 μg ml^{-1} respectively) and 2 (0.74 ± 0.03 and 0.67 ± 0.02 μg ml^{-1} respectively) indicating increased requirement of Mn in adult cattle to carry out various productive and reproductive functions properly. Interestingly, based on the minimum plasma critical level of 0.2 μgml^{-1} (McDowell *et al.* 1984)100 % percent Mn deficiency was observed in the calves of both zone1 (0.14 ± 0.02 μgml^{-1}) and 2 (0.18 ± 0.03 μgml^{-1}). Consumption of dam's milk might have precipitated this deficiency in calves as high Ca and P contents of milk might interfere with Mn absorption in gut (McDowell 1992).

Based on minimum critical level of 0.65 μgml^{-1} (McDowell *et al.* 1984) Cu deficiency could hardly be observed in the adult animals. However, most of the samples collected from the calves, particularly those from zone-2 were deficient (0.66 ± 0.05 μgml^{-1} in zone-1, 0.53 ± 0.03 μgml^{-1} in zone-2). Higher ($P < 0.05$) plasma Cu was observed particularly in the lactating (1.59 ± 0.05μg ml^{-1} in zone-1, 0.83 ± 0.04 μgml^{-1} in zone-2) and dry animals (1.33 ± 0.10 μgml^{-1} in zone-1, 0.85 ± 0.02 μgml^{-1} in zone-2) and this corroborates the findings of Rajora and Pachauri (1993) and Das *et al.* (2002). Cu is a specific activator of some of the important enzyme system viz. cytochrome oxidase, monoamine oxidase, tyrosinase etc. which are required for normal activities of hypophysical

hormones (McDowell 1992). As activity of these hormones increases with age and productive stage a higher plasma Cu is expected in the animals of higher age group and this might be the plausible explanation of the present findings. Moreover, plasma Cu was lower ($P < 0.05$) in animals of zone-2 irrespective of physiological category. Perhaps the better Cu profile observed in the soil, feeds and fodder of zone-1 was reflected in the plasma though it is not fully correct to assess the plasma Cu level on the basis of Cu concentration of feeds and fodder (Rojas *et al.* 1993).

Though the actual plane of nutrition in the grazing bullocks of these agroclimatic zones is yet to be ascertained, the plasma concentrations of major and trace elements did not reveal any serious deficiency in this category of animals. However, as per the adopted feeding regime in the surveyed area, bullocks had to thrive on grazing and paddy straw with least access to any concentrate supplement. Therefore it will be improper to assess the status of mineral nutrition in bullocks only on the basis of plasma concentration.

Trace elements in vital organs

Concentrations of Fe, Zn, Cu and Mn in liver, kidney and lungs showed little variation with agroclimatic zones (Table 4). Liver Fe was much higher than the critical level of 180.0 ppm in both zone-1 (372.8 ± 1.90 ppm) and zone-2 (374.0 ± 2.01 ppm) indicating adequate Fe intake. The present study has therefore further confirmed the earlier reports (Tejada *et al.* 1987, Valdes *et al.* 1988, Knebusch *et al.* 1988) that Fe deficiency is least likely to occur in grazing ruminants of tropics.

None of the vital organ samples was deficient in Zn in either of the agroclimatic zones. The Zn concentration in liver (zone-1 = 114.5 ± 1.18 ppm, zone-2 = 108.6 ± 0.80 ppm) was comparable to the findings of Mtimuni *et al.* (1990) and Cardose *et al.* (1999). reports related to Zn concentration of lungs are scanty and the range of values observed in the present investigation was almost identical to that of Kolacz *et al.* (1994).

Table 4. Trace element contents of vital organs[a]

Trace element	Agroclimatic zone	Vital organs: Liver[b]	Kidney	Lung
Fe ppm	Zone-1	372.8 ± 1.90	284.7 ± 7.10	333.7 ± 6.70
	Zone-2	374.0 ± 2.01	275.2 ± 2.50	331.9 ± 8.60
Zn ppm	Zone-1	114.5 ± 1.18	44.9 ± 0.79	71.1 ± 0.14
	Zone-2	108.6 ± 0.80	40.6 ± 0.41	76.2 ± 0.21
Cu ppm	Zone-1	43.4 ± 0.44	33.6 ± 0.25	28.0 ± 0.20
	Zone-2	40.5 ± 0.45	31.3 ± 1.00	28.8 ± 0.76
Mn ppm	Zone-1	10.5 ± 0.15	8.5 ± 0.22	9.1 ± 0.65
	Zone-2	10.2 ± 0.32	8.5 ± 0.50	8.9 ± 0.49

[a] Based on observation of 12 samples (6 from each zone). [b] Critical level: Fe < 180.0 ppm, Cu < 75.0 ppm, Zn < 84.0 ppm, Mn < 6.0 ppm (McDowell *et al.* 1984)

In both the agroclimatic zones liver Cu (ppm) was lower than the critical level of 75.0 ppm (zone-1 = 43.4 ± 0.44, zone-2 = 40.5 ± 0.45). Since the status of Cu in animals can be ascertained properly only through level of liver Cu therefore the present findings indicate towards the utmost need of Cu supplementation in grazing cattle. It has been reported earlier (Valdes *et al.* 1988, Knebusch *et al.* 1988) and has been confirmed in the present investigation that inadequacy of Cu in diet resulted in depletion of Cu storage in the liver. As a result the animals becomes prone to Cu deficiency despite an apparently normal blood Cu level.

Mn concentration in liver was within the normal range of variation in cattle (zone-1 = 10.5 ± 0.15 ppm, zone-2 = 10.2 ± 0.32 ppm) and the values corroborate earlier reports (Mtimuni *et al.* 1990, Prabowo *et al.* 1990, Cardose *et al.* 1999). Mn in the kidney and lungs were also fairly constant in both the agroclimatic zones. According to CMN (1973) liver is the most promising practical criterion for assessing Mn status of animals. Therefore these data suggest that Mn is not expected to be limiting under the present condition. Nevertheless, the low levels of Mn, particularly in the paddy straw of both the agroclimatic zone, would suggest the need for providing supplemental Mn to the grazing cattle population.

The present investigation has clearly indicated that the grazing ruminants of the new alluvial and red laterite agroclimatic zones were very much prone to mineral deficiency particularly that of Ca, P, Zn and Cu. Paddy straw, one of the main sources of bulk to the livestock of these agroclimatic zones were severely deficient in Ca and P. On the other hand, Cu and Zn were limiting in the pasture grass and other green roughage. Though the plasma concentrations of these major and trace elements were within the normal range of variation liver biopsy indicated that cattle of both the agroclimatic zones had Cu deficiency. The present investigation has emphasised that in the red laterite and new alluvial agroclimatic zones of West Bengal, where the livestock do not receive any mineral supplement, the cattle rations are needed to be supplemented with specific mineral elements to augment the productivity of the grazing livestock.

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Effect of phytase supplementation in maize based diet on growth performance and nutrients utilization of broiler chickens*

P K SINGH[1], V K KHATTA[2] and R S THAKUR[3]

CCS Haryana Agricultural University, Hisar, Haryana 125 004 India

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ABSTRACT

A biological trial was conducted to study the effect of phytase supplementation on the performance of broiler chickens fed maize based rations containing different levels of non-phytate phosphorus (NPP). Day-old broiler chicks (n=240) were divided into 12 groups of 20 birds in each. Four dietary treatments viz. T_1 (control, NPP 0.50%), T_2 (NPP 0.30%), T_3 (T_2 + phytase) and T_4 (T_1 + phytase) were formulated. Each dietary treatment was offered to 3 groups of birds during their starter (0-4 weeks) and finisher phases (4-6 weeks). There was significant ($P \leq 0.05$) depression in growth and feed intake in birds received diet without supplemental phosphorus (T_2) but body weight gains, feed intake and feed conversion efficiency improved significantly ($P \leq 0.05$) on supplementation of phytase (T_3). Dry matter metabolisability and retention of nitrogen, calcium and phosphorus improved significantly ($P \leq 0.05$) in diets supplemented with phytase. However, phytase supplementation to high NPP (0.50%) diets didn't improved ($P \leq 0.05$) the performance of broilers. The supplementation of phytase on low NPP diet increased concentration of serum phosphorus, tibial ash and its calcium and phosphorus content. The results indicated that supplementary phytase was beneficial in diets without inorganic phosphorus supplementation (0.30% NPP).

Key words: Broiler chickens, Maize, Non-phytate phosphorus (NPP), Phytase

Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) is a naturally occurring organic complex in cereals and legumes that present upto a level of approximately 5% by weight on dry matter basis (Tyagi *et al.* 1998), which in turn binds 50-80% of the total phosphorus present (Ravindran *et al.* 2000). Under most dietary conditions, the phytate bound phosphorus and other nutrients are either unavailable or poorly utilized by poultry (Nelson 1967). Phytase enzyme is present in very low concentrations in the small intestine of chicken (Maenz *et al.* 1997) and not sufficient to improve availability of phytate P. Phytase is also known to be produced by some fungi, bacteria, yeast and rumen microorganisms. The microbial phytase has broad pH activity range and, more effective in the gastrointestinal environment of poultry. The microbial phytase has provided a practical way to improve availability of phytate bound P (Eeckhout and De Paepe1994). Maize, the major energy source of poultry diet, contains 0.25% phytate phosphorus, accounting for 64% of the total P (Tyagi *et al.* 1998). Therefore an attempt has been made to evaluate the performance of broilers fed maize based ration supplemented with phytase in the present study.

MATERIALS AND METHODS

Day-old commercial broiler chicks (240) were randomly distributed to 4 treatment groups consisting of 3 replicates of 20 birds in each. The birds were raised on litter upto 6 weeks of age at 2 phases of feeding i.e. starting (0-4 weeks) and finishing (4-6 weeks) under similar managemental conditions. Four isocaloric and isonitrogenous diets containing 23% crude protein (CP) and 2800 Kcal/kg metabolisable energy (ME) during starting and 20% CP and 2900 Kcal ME/kg during finishing period were formulated as per BIS (1992). The dietary treatments (Table1) were T_1 (maize based ration supplemented with inorganic phosphorus, 0.50% NPP), T_2 (maize based ration without phosphorus supplementation 0.30% NPP), T_3 (T_2 + phytase), T_4 (T_1 + phytase). The phytase was added @ 30 g/100 kg of broiler diets to provide 750 phytase units (FYT) activity/kg diet. Each dietary treatment was offered *ad lib.* to 3 replicated groups of broilers. Feed ingredients used (maize, rice polish, deoiled rice polish, groundnut-cake, soybean-meal and fish-meal) were analyzed for proximate nutrients, calcium, total phosphorus (AOAC

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Present address: [1]Ph. D. Scholar, Division of Animal Nutrition, IVRI, Izatnagar, India 243 122. [2]Associate Professor, Department of Animal Nutrition, [3]Professor, Department of Animal Nutrition, College of Animal Sciences.

Table 1. Ingredient and chemical composition of experimental diets

Ingredients/supplements (100 kg)	Starter mashes (0-4 weeks)				Finisher mashes (4-6 weeks)			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Maize	40	40	40	40	52	52	52	52
Soyabean-meal	20	20	20	20	14	14	14	14
Groundnut cake	11	11	11	11	10	10	10	10
Rice polish	12	12	12	12	10	10	10	10
Deoiled rice polish	8.7	9.2	9.2	8.7	5.6	6.1	6.1	5.6
Fish-meal	6	6	6	6	6	6	6	6
Dicalcium phosphate	1.1	-	-	1.1	1.2	-	-	1.2
Calcium carbonate	0.9	1.5	1.5	0.9	0.9	1.6	1.6	0.9
Others[1]	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Lysine	0.05	0.05	0.05	0.05	-	-	-	-
DL-methionine	0.07	0.07	0.07	0.07	-	-	-	-
Phytase[2]	-	-	0.03	0.03	-	-	0.03	0.03
Chemical compositiion (On air dry basis)								
Metabolizable energy (Kcal/kg)*	2800	2812	2812	2800	2900	2911	2911	2900
Crude protein (%)**	22.91	22.99	22.99	22.91	20.10	20.17	20.17	20.10
Crude fibre (%)**	5.93	5.99	5.99	5.93	5.08	5.15	5.15	5.08
Calcium (%)**	1.21	1.20	1.20	1.21	1.20	1.21	1.21	1.20
Total phosphorus (%)**	1.00	0.82	0.82	1.00	0.95	0.76	0.76	0.95
Phytate-phosphorus (%)**	0.50	0.51	0.651	0.50	0.45	0.46	0.46	0.45
Non-phytase-phosphorus (%)**	0.50	0.31	0.31	0.50	0.50	0.30	0.30	0.50
Lysine (%)*	1.20	1.20	1.20	1.20	1.00	1.01	1.01	1.00
Methionine (%)*	0.50	0.50	0.50	0.50	0.38	0.38	0.38	0.38

*Calculated values; **Analysed values; [1]Others include common salt (300g), Avet TM (75 g), Indomix BE (10 g), Indomix$A+B_2+D_3+K$ (25g), Cygro (50 g) and Lincomix (10g). [2]Each gram phytase contained phytase activity of 2500 FYT (One FYT is the amount of enzyme that releases 1m mol inorganic phosphate/ min from 5.0 mM sodium phytase at pH 5.5 and 37°C).

1995) and phytate-phosphorus (Haugh and Lantzsch 1983).

The average body weight gain and average feed intake per bird were recorded on 2nd, 4th and 6th weeks of age and feed conversion ratio were calculated for all treatment for the same growth periods. A metabolism trial of 3 days was conducted during 6th week of growing periods for the evaluation of retention of nitrogen, calcium and phosphorus. Data of feed intake, excreta voided, dry matter content of feed and excreta were recorded and estimated. The samples of feed and excreta were analysed for nitrogen, calcium and phosphorus (AOAC 1995) and retentions were expressed as proportion of the nutrients retained per unit weight of intake. Six birds per treatment (2 birds per replicate) were slaughtered after 6 weeks of age by cervical dislocation to collect blood samples and left tibia. Blood samples were analyzed for serum calcium (Gitelman1967) and inorganic phosphorus (Fiske and Subbarow 1925). The concentrations of calcium and phosphorus were expressed in mg/day. Tibial were freed of soft tissue and defattened by soaking in petroleum ether for 48 hr. The dried bone samples were ashed at 600°C for 12 hr. Total ash and P(AOAC 1995) and Ca (Talpatra 1940) content of tibial bone were analysed. Statistical analysis of data was done using Duncan multiple range test (DMRT) as modified by Kramer (1956).

RESULTS AND DISCUSSION

Growth performance, feed intake and feed efficiency is presented in Table 2. Body weight gain of the broilers depressed due to reduction in NPP from 0.50 to 0.30% with dietary calcium maintained at 1.20% level. It is evident that maintaining Ca : NPP ratio (2.4:1) results in better performance of broilers. Supplementation of phytase (750 FY kg diet) significantly improved ($P \leq 0.05$) the body weight gain of chicks. However, no significant improvement was observed due to its supplementation in high NPP diet (0.50%). Similarly feed consumption was significantly ($P \leq 0.05$) reduced when birds were fed low NPP diets as compared to control diet. When low NPP diets was supplemented with phytase, there was a general tendency for the consumption to increase. However, feed intake was not affected by the phytase supplementation to high NPP diet. Feed efficiency was significantly ($P \leq 0.05$) depressed at low NPP levels in diet but improved by the supplementing the diet with phytase. Similar improvement in growth parameters was reported by Sebastian *et al.* (1996) and Rama Rao *et al.* (1999).

The improvement in growth performance of broilers fed phytase might be the result of better bio-availability of nutrients due to hydrolysis of phytate-mineral complex

Table 2. Effect of phytase supplementation on the body weight gain, feed intake and FCR of broilers

	Body weight gain (g/chick)		Feed intake gain (g/chick)		FCR	
Traeatments	0-4 Wks	0-6 Wks	0-4 Wks	0-6 Wks	0-4 Wks	0-6 Wks
T_1	983.23^a±6.66	1764.18^a±13.74	1877.96^a±18.77	4110.60^a±26.37	1.91^a±0.00	2.33^a±0.02
T_2	817.99^b±11.36	1426.58^b±14.43	1644.16^b±17.86	3380.99^b±28.87	2.01^b±0.01	2.37^a±0.01
T_3	933.95^c±12.40	1680.00^c±23.58	1709.12^c±14.71	3561.60^c±39.57	1.83^c±0.01	2.12^b±0.01
T_4	990.06^a±13.89	1769.22^a±26.77	1900.92^d±24.94	4141.60^a±16.05	1.92^a±0.01	2.34^a±0.01

Values in a column bearing different superscripts differ significantly (P≤0.05).

Table 3. Effect of different treatments on the retention of dry matter, nitrogen, calcium and phophorus, serum calcium and phosphorus, tibial ash and its calcium and phosphorus content

Treatments	DM metabo-lisability (%)	N retention (%)	Ca retenition (%)	P retention (%)	Serum Ca (%)	Serum P (%)	Tibial ash (%)	Tibial Ca (%)	Tibial P (%)
T_1	66.50^a±0.33	53.54^a±0.78	42.81^a±0.73	60.40^a±0.77	11.20±0.44	5.86^b±0.13	50.44^a±0.03	35.35^a±0.20	16.89^a±0.57
T_2	70.08^b±0.21	54.12^a±0.16	40.75^a±0.10	56.67^b±0.58	10.31±0.36	4.26^a±0.26	43.17^b±0.68	35.32^a±0.23	15.50^b±0.25
T_3	71.65^c±0.83	58.26^b±0.74	44.78^b±1.12	65.80^c±0.47	11.46±0.26	5.14^b±0.07	47.57^c±0.21	36.78^b±0.39	17.07^a±0.19
T_4	71.80^c±0.62	59.18^b±0.72	42.70^a±0.44	61.45^a±0.52	10.80±0.42	5.69^b±0.50	51.56*±0.72	37.08^b±0361	17.75^a±0.24

Values in a column bearing different superscripts differ significantly (P≤0.05).

(Simons *et al.* 1990; Sebastian *et al.* 1996) or the utilization of inositol by birds after hydrolysis of phytic acid to inositol (Simons *et al.* 1990). The effects of phytase supplementation on nutrient utilization have been presented in Table 3. An increase in phosphorus retention at low dietary NPP may be explained by homeostasis mechanism of body that increases retention and absorption of phosphorus at low dietary intake as compared to normal dietary phosphorus (Allen and Wood 1994). Phytase supplementation in the low dietary NPP diet (0.30%) significantly (P≤0.05) increased dry matter metabolisability, nitrogen, calcium and phosphorus retention by hydrolyzing phytate to release phytate bound phosphorus, protein and other compounds and thus increasing the digestion and absorption of nutrients (Ravindram *et al.* 2000, Ahmad *et al.* 2000). The value of serum calcium in different treatments did not differ significantly (P≤0.05). Similar findings were also reported by Sebastian *et al.* (1996). Serum inorganic phosphorus significantly reduced (P≤0.05) in birds fed low NPP diets. The low serum phosphorus levels resulting from low dietary intake were increased (P≤0.05) due to phytase supplementation. Ash, calcium and phosphorus content of tibia significantly (P≤0.05) increased as the low NPP diets were supplemented with phytase.

A similar result was reported by Simons *et al.* (1990), Sebastian *et al.* (1996) and Rama Rao *et al.* (1999). Improved tibial ash and its mineral contents may be attributed to better bone mineralization as a result of hydrolysis of phytate-mineral complex by phytase which in turn increases calcium and phosphorus concentration (Ahmad *et al.* 2000).

The result of this experiment indicated that phytic acid is a potent anti-nutritional factor that can depress the performance of broilers. It was also concluded that phyase supplementation (750FYT/kg diet) to a practical broiler diet containing low NPP (0.30%) could partially overcome the adverse effect of phytic acid and might reduce usual supplementation of inorganic source of phosphorus.

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Backyard poultry in Meghalaya

N PRAKASH[1], RAJESH KUMAR[2] and P P PAL[3]

ICAR Research Complex for NEH Region, Umiam, Meghalaya 793 103 India

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ABSTRACT

In Meghalaya backyard poultry is taken up in its traditional way though it has immense scope for improvement. A study among 120 tribal farmers on existing poultry rearing practices has shown that the tribals need adequate training for scientific rearing. Government support, institutional support and proper marketing structure are badly needed to make this venture in to a profitable one. The chicken and egg can be an ideal alternative/substitute for meat requirement of the tribal people of Meghalaya, as well as it can create additional employment opportunities provided this sector receives proper attention from all concerned.

Key word: Poultry development

The tribals of Meghalaya being non-vegetarian in their food habit consume different types of meat in their daily diet. Among various meat, beef, pork, mutton and chicken are commonly found in their daily diet. Accordingly, tribals rear these livestock in their own way and small holder livestock production systems dominate in this state due to many reasons. Small holder backyard poultry production makes minimum use of land, labour and capital (Proceeding of Seminar 2000). A study was conducted among 120 tribal farmers of Meghalaya to assess the existing backyard poultry rearing practice and to find out the scope for its improvement. The objectives of study were: to study the socio-economic status of the poultry farmers of Meghalaya, to highlight the existing poultry rearing practices, to find out different reasons behind the existing rearing practices, and to delineate different constraints faced by the poultry farmers.

MATERIALS AND METHODS

Three districts from Khasi Jaintia and Garo Hills were purposively selected for this study. From each district 2 villages were selected randomly. From these 6 villages 20 farmers again were selected by simple random sampling technique. The list of the farmers having backyard poultry was taken as the prime criterion in preparing the list. Thus 120 farmers were selected who were rearing backyard poultry for at least 5-6 years. Standard statistical tools and percentage statistics were used to calculate the data. The data were collected by a pre-tested questionnaire and personal observation methods.

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Present address: [1]Incharge, [2,3]Scientist, Division of Agricultural Extension.

RESULTS AND DISCUSSION

Poultry population trend of Meghalaya

As per information published by North Eastern Council through Basic Statistics of North Eastern Region 2000, a total poultry population (improved and *deshi*) has shown a positive trend in Meghalaya. The total poultry population of Meghalaya in 1992 has been estimated as 1824 thousand from the population of 975.3 thousand in the year 1972. Though there has been increase in population of about 200% still the per capita availability of eggs per year is estimated at 58 numbers and the total number of eggs brought from outside the state is estimated at about 32 million numbers in a year (Anon 1997, status paper prepared by State Government of Meghalaya and Indian Poultry Industry Year Book 1994).

The socio-economic profile of the selected farmers was studied to get an insight about the general socio-economic condition of the farmers. However, the details of socio-economic profile of the selected poultry farmers are presented in Table 1.

Most of the farmers (61.66%) are illiterate, belong to middle aged category (70.83%), having medium to small family size, low to medium annual income and very low (50.83%) social participation. This implies that the farmers who really cannot afford any other form of poultry rearing stick to small holder backyard poultry farming

Table 1. Socio-economic profile of the poultry farmers

Variables	Category	Frequency	Percentage
Age	Young (25-30 year)	23	19.16
	Middle (31-35 year)	85	70.83
	Old (35 and above)	12	10.00
Education	Illiterate	74	61.66
	Up to primary	34	28.33
	High schools and above	12	10.00
Family size	Small (3-5 members)	36	30.00
	Medium (6-8 members)	49	40.83
	Large (more than 8 members)	15	12.50
Annual income	Low (less than Rs 10 000)	62	51.66
	Medium (less than Rs 15 000)	58	48.33
	High (Rs16 000 and above)	10	8.33
Social participation	Low	61	50.83
	Medium	21	17.50
	High	38	31.67
Livestock possession	Cattle and buffalo (Nos)	42	35.00
	Pig (Nos)	110	91.67
	Poultry (Nos)	120	100.00
	Goat (Nos)	58	48.33

Existing poultry rearing practices

The different poultry rearing practices were observed to have an idea about the traditional method of poultry rearing in 3 hills of Meghalaya (Table 2).

Disease management and local marketing system are the most prominent aspect (Table 2) of poultry rearing in Meghalaya. Local market means house-to-house disposal as per the household need.

Poultry as commercial venture is hardly practised in the study area followed by no definite rearing period (78.33%), housing (85.00%) and non-supplement of any additional feed (60.00%). No formal training in poultry rearing has been received by 70.00% of the farmers and non-casing rearing i.e. keeping of birds in a single basket during night is very common in the study area.

Reasons behind traditional rearing

The farmers were asked to enumerate reasons behind such traditional method of rearing and reasons narrated by them are presented in Table 3.

Table 2. Traditional poultry rearing practices

Observations	Frequency	Percentage
No definite rearing period	94	78.33
No additional feed	72	60.00
No disease management practices	120	100.00
Non-casing rearing	88	73.33
No formal training	84	70.00
No proper housing	102	85.00
No commercialization	95	79.16
Local marketing system	120	100.00

Table 3. Reasons for traditional rearing of poultry birds

Reasons	Frequency	Percentage
Lack of knowledge	52	43.33
Lack of institutional support	31	25.83
Liking for taste	60	50.00
Unorganized marketing sector	27	18.33
Lack of hatchery facility	42	35.00
Non availability of chicks	52	43.33
Less risk bearing ability	62	51.66
Lack of Government support	79	65.83
Lack of feed ingredients	66	55.00
Lack of storage facility	47	39.16

Lack of government support has been the key factor behind non-improvement of poultry sector as indicated by 65.83% of farmers (Table 3). This has again accentuated by lack of feed ingredients and less risk bearing ability of the farmers (55.00% and 51.66% respectively). Lack of knowledge about improved poultry rearing and non-availability of quality chicks are another 2 important reasons mentioned by the farmers. The reasons are quite obvious as the farmers mostly belong to lower socio-economic strata and they can hardly improve poultry rearing of their own. Simultaneously they are totally dependent on State Government for formulation of approriate policies and rendering assistance for poultry improvement.

Constraints faced by the farmers

Different constraints have been delineated by the farmers regarding poultry rearing which are presented in Table 4.

Perusal of data of (Table 4) revealed that infrastructural facility is the urgently felt need by most of the farmers (86.66%) followed by credit facility and lack of training (85.00% and 76.66%). The other important constraints indicated by the farmers are absence of cooperative system, lack of government policy and non-existence of feed industry. The findings are quite encouraging in the sense that in spite of poor economic condition and low education level and social participation, the farmers are quite interested for the improvement of this sector to improve their own economic

Table 4. Constraints faced by the poultry farmers (120)

Constraints	Frequency	Percentage	Rank
Infrastructural facility	104	86.66	I
Government policy	64	53.33	V
Support system	52	43.33	VIII
Cooperatives system	72	60.00	IV
Feed industry	62	51.66	VI
Training of farmers	92	76.66	III
Credit facility	102	85.00	II
Marketing	51	47.00	VII
Transportation	42	35.00	IX

condition through this venture.

It may be concluded that the socio-economic conditions of the tribal farmers are quite low and poultry rearing is very popular in the rural side of Meghalaya. Poultry rearing is confined as smallholder backyard poultry only and no support system has been provided to the poultry farmers. Storage facility for egg and chicken are badly needed. Total improvement of this sector needs proper planning, creation of adequate infrastructure and monetary support.

To make backward poultry rearing a profitable venture the farmers should be adequately trained in scientific poultry rearing. The availability of feed and quality chicks may also be assured by the Government Agencies. The financial organizations should also extend their support to make it a lucrative sector that has immense scope of additional income generation.

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Growth response of West African dwarf lambs fed yam tuber peel

N M ANIGBOGU[1]

University of Agriculture, Umudike, P.M. B 7267, Umuahia, Abia State, Nigeria

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Yam (*Dioscorea* spp.) is among the staple energy food crop of most people in the tropics and one of the importance root tuber crops of West African (Nweke *et al.* 1991). About 4.18-4.78 million metric tonnes of the byproduct (yam tuber peel) are globally available for feeding ruminants, especially sheep. While in Nigeria the yam production is about 3.14 to 3.59 million metric tonnes (Asiedu 1994). Yam tube peel can serve as an important source of energy in ruminant feeding systems, serving either as the basal diet or as supplement. It follows that, poor nutrition during the long dry season of the year (Larsen and Amaning-kwarteng 1976) is among the main constrain to livestock production in Nigeria, which is a major yam producing country in the world. There is considerable livestock weight loss and sometime death which are caused by the rapid decline in the quality and quantity of forages (Otchere *et al.* 1977), that lead to poor intake and digestibility. In Nigeria there is a need to explore yam tuber peel as an alternative feed for livestock which industries producing yam floor, yam fufu or yam chips throws out as waste and is scavenged by sheep on rubbish dumps in villages and urban areas. The by-product is slightly richer in protein, fiber, ash, fat and lower in carbohydrate than the tubers, thus the use of yam tuber peel necessitates a protein supplement to adjust the ration to the animals requirements. Information is lacking on intake and utilization of yam tuber peel as supplement feed when fed to sheep. The present study was therefore conducted to assess the potential of sun-dried yam tuber peels as a component of forage in sheep feeding during the dry period of the year.

The yam tuber (mixed species) peels gathered from homes and industrial establishments were sun-dried for 2-4 days (Anigbogu 1997), before use as a component of the rations. In the study, 3 yam tuber peel based rations were formulated. Grasses and legumes (2 : 1) of mixed species were processed into hay and the trial rations were prepared as follows: A (70% hay), B (35% hay + 35% yams tuber peel), and C (70% yam tuber peel). Cotton seed-cake and palm kernel-cake were incorporated at 15% each as protein supplement for the rations. The hay was chopped into bits, mix with yam tuber peel, cotton seed-cake and palm kernel-cake, then given *ad lib.* to the experimental lambs and water provided at free choice. An adjustment period of 14 days was allowed before measurements were taken. Twenty-seven male lambs (average = 4.5 weeks) were reared (average floor area = 0.60m^2 per lambs) for 77 days in an empty broiler-litter house that was naturally ventilated. Wood-shaven was spread in the pens as bedding material for the lambs. Lambs were randomized to 9 pens of 3 lambs each with an average pen weight of 25.2 kg and a maximum difference between pens of 0.21kg. The data on fed intake, weight gains, feed conversion efficiency, cost of feed input/kg live weight gain and dressing percentage of the lambs were compared by duncan's multiple range Test (Duncan 1955) and (Gomez and Gomez 1984).

Initial weight was similar in the groups (8.4 kg) while the finishing weight and average daily gain in the experiment were higher in C followed by B and A groups (Table 1). The result are in conformity with the findings of Gohl (1981), as the yam tuber peel is highly degradable source of carbohydrate in the rumen (Smith *et al.* 1992). The feed to gain ratio was significant (<0.01) narrower in 70% yam tuber peel fed lambs than the other 2 groups. The feed conversion efficiency of the group was about 20%, which will be considered optimum for sheep in active phase of growth. The improve feed conversion efficiency of lambs fed yam tuber peel was due to high content of soluble carbohydrate in the feed source. The findings are in argument with observations of Gohl (1981) and Smith *et al.* (1992) in cattle, sheep, goats and pigs fed yam or cassava tuber peels.

The dressed carcass weight in the 3 groups was highest in C (13.05 kg) followed by B and A groups respectively. This is in agreement with the suggestion made by Anigbogu (1990), as yam tuber peel is a well utilization source of carbohydrate in the rumen system of sheep, Hahn *et al.* (1992) also observed increase in dressed carcass weight which is associated with a significant increase in live weight gain. However, the dressing percentage on empty live weight was not significant (P>0.05), the dressing percentage on pre slaughter weight also followed

Present address: [1]Department of Animal Nutrition and Feedmilling Technology, Michael Okpara.

Table 1. Feed intake, body weight gain, feed efficiency, cost of feed input/kg live weight gain, and dressing percentage of West African dwarf lambs fed yam tuber peel based rations

Traits	A	B	C
Initial weight (kg)	8.40	8.40	8.40
Final weight (kg)	11.70[a]*	16.40[b]	25.60[c]
Total gain (kg)	3.30[a]	8.00[b]	17.20[c]
Average daily gain (g)	43.00[a]	104.00[b]	223.00[c]
Total feed consumed/lamb (kg)	71.29[a]	111.64[c]	88.01[b]
Feed efficiency (kg feed/kg gain)	21.47[a]	13.99[b]	5.12[c]
Feed to gain ratio	6.10[a]	6.80[a]	3.40[b]
Dressed carcass weight (kg)	6.09[a]	8.44[b]	13.05[c]
Dressing % on pre slaughter weight	52.05	51.46	50.98
Dressing % on empty live weight	63.50	62.75	62.17
Cost of feed input/kg live weight (N)[1]	479.80[a]	274.29[b]	197.27[c]

*Unlike superscripts in a row differ significantly (P<0.01); [1]N=Nigerian currency Naira, Nigerian N 140=US $1=Indian Rs 50.

the same trend with about 52%, which can be regarded high for sheep in practical operation of growth. The lower dressing percentages of the lambs fed yam tuber peels as noted in this study was as a result of high degradable carbohydrate and lower fibre present in the major feed source. The findings are in agreement with the revelations of Smith *et al.* (1988), Anigbogu (1990) and Hahn *et al.* (1992) in cattle, goats and sheep fed tuberous root byproduct including yam tuber peel. The costs of feed input/kg gain live weights of the lambs were lower in C follow by B and A groups respectively. This was significant (P<0.01) closely confine in 70% yam tuber peel fed lambs than other 2 groups. The observation is in correspondence with the findings of Little and Said (1986), as yam tuber peel is a relatively cheep feed source with highly degradable carbohydrate source for lambs (Ahmed 1977, Gohl 1981, Smith and Bosman 1987)

SUMMARY

The potential of sun-dried yam tuber peels as a component of forage in sheep feeding during the dry period of the year, was studied. The study revealed that the yam tuber peel can serve as an important feed source in sheep to improve the low quality nutrition during long drier months.

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Factors affecting economic traits in crossbred cattle

JUNED AKHTER[1], HARPAL SINGH[2], D KUMAR[3] and K SHARMA[4]

Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttaranchal 263 145 India

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Key words: Crossbred cattle, Efficiency traits, Genetic groups, Period effect, Production traits, Reproduction traits, Season effect

Performance of various genetic grades of crossbred cattle for first lactation traits and effects of some non-genetic factors thereon have been studied under this investigation.

Data used in this study pertained to 563 two- and three-breed cross progenies from 38 sires of 3 exotic breeds viz. Holstein Friesian (HF), Jersey (J) and Red Dane (RD) with 3 zebu breeds, viz. Sahiwal (S), Hariana (H) and Red Sindhi (RS) constituting 12 genetic groups, maintained at Livestock

Table 1. Least-squares means and constants for various genetic groups, periods and seasons of first lactation traits in crossbred cattle

Factors	No. of obs.	Traits						
		FLMY (kg)	FLP (days)	FDP (days)	AFC (days)	FCI (days)	FLY/DLP (kg/day)	FLY/DCI (kg/day)
μ	563	2374.23±58.72	306.77±2.15	85.59±2.69	1040.19±14.06	392.63±2.59	7.75±0.08	6.09±0.08
Genetic groups								
5/8 F×3/8 S	259	621.02	14.26	-20.28	30.49	-6.01	1.66	1.73
1/2 F×1/2 S	30	209.24	0.57	-14.50	36.42	-13.93	0.68	0.79
1/2 R×1/2 S	09	-0.99	8.73	-20.73	-54.64	-11.99	-0.20	0.21
5/8 J×3/8 S	16	155.72	17.64	-16.92	-1.93	0.72	0.07	0.43
1/2 F×1/2 S	09	-0.78	17.80	-19.57	-81.98	-1.76	-0.39	0.11
1/2 F×1/2 H	96	7.75	-12.44	0.004	69.86	-12.43	0.31	0.20
J×R.S.	65	30.05	-0.43	0.98	-114.47	0.55	0.12	0.07
J (R×S)	24	-269.10	28.40	26.12	2.28	-2.26	-0.13	-0.70
F (J×S)	12	172.23	1.53	7.81	-60.70	9.34	0.51	0.25
F (R×S)	14	-178.23	-10.45	10.27	59.11	-0.18	-0.37	-0.53
J (F×S)	09	-416.95	-19.05	26.10	46.06	7.05	-0.93	-1.23
>75% exotic inheritance	20	-329.96	-46.56	20.72	68.50	30.90	-1.33	-1.33
Period								
P_1 (1965-68)	28	-386.11	-16.98	27.11	40.29	10.12	-0.84	-0.12
P_2 (1969-72)	96	-21.10	-12.59	11.70	36.21	-0.91	-0.37	-0.57
P_3 (1973-76)	130	-9.77	7.39	-4.70	-23.79	2.71	-0.22	-0.78
P_4 (1977-80)	99	111.83	8.08	-9.03	33.15	-0.95	0.17	0.34
P_5 (1981-84)	159	276.62	14.06	-13.08	19.43	1.04	0.56	0.76
P_6 (1985-88)	51	28.53	0.04	-12.07	47.71	-12.01	0.70	0.37
Seasons								
Spring (Jan-Mar)	147	-1.35	-4.06	-2.33	5.03	-6.94	-0.16	-0.12
Summer (Apr-June)	136	-41.89	0.37	5.46	-24.09	5.84	-0.92	-0.40
Rainy (July-Sept)	150	20.74	-1.20	-4.02	19.46	-4.68	0.35	0.11
Winter (Oct-Dec)	130	22.50	4.89	0.89	-0.40	5.78	0.73	0.41

Present address: [1]Scientist, B K V K, Fatehpur, Shekhawati, District Sikar, Rajasthan.

Professor and Head, [3]Associate Professor, [4]Assistant Professor, Department of Animal Science, College of Agriculture.

Research Centre of the University and 4 military dairy farms viz. Agra, Ambala, Bareilly and Meerut. The performance records on first lactation milk yield (FLMY), first lactation period (FLP), first dry period (FDP), age at first calving (AFC) first calving interval (FCI), first lactation milk yield per day of first lactation period (FLY/DLP) and first lactation milk yield per day of first calving interval (FLY/DCI) spreading over a period of 24 years from 1965 to 1988 were studied.

The records were classified according to 12 genetic groups, 6 periods of calving/birth of 4 years each and 4 sessions viz. spring, S_1 (January to March), summer, S_2 (April to June), rainy, S_3 (July to September) and winter, S_4 (October to December) to study their effects on the above first lactation traits of crossbred cows. In order to obtain the effects of these factors and to make orthogonal comparisons, least squares analysis by fitting constants as per Harvey (1985) was carried out.

The least-squares means for FLMY, FLP, FDP, AFC, FCI, FLY/DCI traits in crossbred cows were estimated as 2374.23±58.72 kg, 306.77±2.15 days, 85.59±2.69 days, 1040.19±14.06 days, 392.63±2.59 days, 7.75±0.08 kg/day and 6.09±0.08 kg/day, respectively, which were all in the range reported in the literature.

The effects of genetic groups were significant for all the traits except FCI. Least squares constants (Table 1) revealed that 5/8 F × 3/8 S crossbreds had produced highest while J (F×S) crossbreds produced lowest milk in their first lactation. The cows with 62.5% exotic inheritance from Friesian or Jersey and 37.5% from Sahiwal had higher FLMY, longer FLP, shorter FDP, lower AFC and FCI and higher FLY/DLP and FLY/DCI than their own half-breds and all the 3 breed crosses. Friesian crosses were superior to all Jersey crossbreds. Further, 3 breed crosses except F (J×S) showed lower FLMY compared to the 2 breed crosses and cows with 75% of exotic inheritance had comparatively lower FLMY, FLP and higher FDP, AFC and FCI than any other level of exotic inheritance. This could be due to the poor adaptability of higher exotic inheritance to the hot/humid hot climatic conditions of India.

The period effects were significant (Table 1) for FLMY, FDP, FLY/DLP and FLY/DCI and non-significant for FLP, AFC and FCI traits. It is evident from Table 1 that there was an increase in FLMY, FLP, FLY/DLP and FLY/DCI and decrease in FDP, AFC and FCI traits over a period of time (P_1 to P_5) mainly due to an increase in the number of animals with 62.5% exotic Holstein Friesian blood and the use of better bulls with higher genetic potential for these traits. The decrease in FLMY during last period (P_6) was directly associated with decrease in the number of animal of 5/8 F × 3/8 S genetic group and the increase in the number of animals beyond 62.5% exotic inheritance which had poor adaptability to the Indian conditions.

Seasons effects were significant (Table 1) for FLMY, FDP, AFC and FCI traits and non-significant for FLP, FLY/DLP and FLY/DCI traits. Winter calvers produced highest milk followed by rainy, spring and summer calvers. This could be due to the better availability of fodder and best temperature for crossbred cows during winter season compared to other seasons.

SUMMARY

Performance of various genetic grades of crossbred cattle for first lactation traits and effects of some non-genetic factors were studied. For higher FLMY, longer FLP and shorter FDP, 62.5% Holstein Friesian or Jersey with 37.5% Sahiwal inheritance may be recommended. Further 2 breed crosses may be preferred over 3 breed crosses. For shorter FDP the calving should be directed from July to September. However, for higher FLMY and longer FLP calving should be synchronized during the months of October to December.

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Efficacy of clove oil as an anaesthetic agent in fishes

SATISH K SRIVASTAVA[1], N S NAGPURE[2], B KUSHWAHA[3] and A G PONNIAH[4]

National Bureau of Fish Genetic Resources, Canal Ring Road, Telibagh, P.O. Dilkusha, Lucknow, Uttar Pradesh 226 002 India

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Anaesthetics are used in aquaculture to minimize stress and damage to fish during harvesting, stripping, weighing, measuring, sorting, tagging, transportation, vaccination, handling for photography and research, so that they can properly recover from the procedure, and return to normal feeding and growth as soon as possible. Anaesthetics, TMS (tricaine methane sulfonate), finquel (MS-222), marinil (metomidate hydrochloride), 2-phenoxyethanol, carbon dioxide, quinaldine, carbonic acid, benzocaine and methyl-,pentynol (Basavaraju *et al.* 1998, Gelwicks and Zafft 1998; Munday and Wilson 1997) are used in fishes world-wide. Most of the anesthetics are synthetic chemicals and may have adverse effect on human health if such fishes are used for human consumption. Thus an eco-friendly biodegradable anesthetic agent is to be preferred over the chemical agents.

Clove (*Eugenia aromatica,*) oil contains the compound eugenol, and is widely used as topical anaesthetic in human medicine. Clove oil (eugenol) was used as a fish anesthetic by Soto and Burhanuddin (1995),Anderson *et al.* (1997), Munday and Wilson (1997) and Kenee *et al.* (1998) but only recently it has became the focus of research, which is aimed at establishing its effectiveness and safety as an anesthetic for use in both aquaculture, wild capture and laboratory research on coral reef fishes. The anesthetic effect of clove oil on exotic fishes was studied in other countries (Anderson *et al.* 1997, Soto and Burhanuddin 1995, Munday and Wilson 1997, Keene *et al.*1998, Waterstrat 1999), however, a study on this line has not been so far initiated in Indian fishes. In view of above, the present study was undertaken to evaluate the efficacy of clove oil as anesthetic agent in indigenous species *Channa punctatus* and exotic species *Clarias gariepinus,* abundantly available in local market.

Preliminary trials were conducted to determine the approximate dose of clove oil required for anesthetization in fishes, before initiation of experiment. For the present investigation specimens of *C. punctatus* (n=30) and *C. gariepinus* (n=30) were procured from local fish market, Lucknow and acclimatized to laboratory condition for a week. Fishes of each species were divided into 6 groups, each containing 5 specimens. In each group specimens were kept separately in plastic tub containing 5 liters of tap water and their weights were recorded. Specimens of first group were kept in untreated water, which served as control. Fishes belonging to group 2 to group 6 were exposed to 25, 50, 100, 150 and 200 ppm solution of clove oil in water respectively. The fishes were monitored closely and time was recorded from initiation of treatment till fishes completely lost their equilibrium, which was marked by total loss of muscle tone and equilibrium with slow but regular opercular rate. Fishes were then removed from clove oil solution, washed with freshwater and kept in running water for recovery and the time was recorded when fishes maintained equilibrium in the water column and attained normal swimming. The induction time for anesthesia and recovery time was recorded individually for each specimen and then average was calculated for each group. The data were subjected to the analysis of variance (Snedecor and Cochran 1967) and if values were significant critical difference test were applied.

Clove oil was not effective in inducing anesthesia when used in lower dose of 25 ppm, even after prolonged treatment of 2 hr, however,the activity of specimens were sluggish as compared to fishes of control group. At higher concentration of 50,100,150 and 200-ppm clove oil induced anesthesia in fishes at different time durations (Table 1). In both species there was a dose dependent decrease in the induction time with a marked decrease between 100 and 200 ppm. The shortest induction time recorded were 2.198 ± 0.28 min and 1.90 ± 0.22 min in *C. gariepinus* and *C. punctatus*, respectively, which were significantly different ($P<0.01$) from induction time required at lower doses. With regard to recovery time no significant dose dependent response was observed (Table 1) in both the species. In *C. gariepinus* longest recovery time of 6.818 ± 0.406 min was recorded for specimen exposed to 200 ppm, which was not significantly ($P<0.01$) different from other treatment groups except fishes

Present address: [1,2,3]National Bureau of Fish Genetic Resources, Canal Ring Road, Telibagh, P.O. Dilkusha, Lucknow,Uttar Pradesh, 226 002 India [4]International Centre for Live Aquatic Resource Management (ICLARM), P.O. box No. 500 GPO, 10670, Penang, Malaysia.

Table 1. Induction time (mean ± SE) for anaesthesia and recovery time (mean ± SE) in fishes using different concentration of clove oil

	Induction time (min)			
	Dose of clove oil (ppm)			
Species	50	100	150	200
C. gariepinus	24.340[a] ±0.91	9.606[b] ±0.34	4.940[c] ±0.23	2.198[d] ±0.28
C. punctatus	34.340[a] ±0.91	5.546[b] ±0.186	4.100[b] ±0.33	1.900[c] ±0.22
	Recovery time (min)			
C. gariepinus	3.400[a] ±0.28	5.666[b] ±0.214	6.430[b] ±0.31	6.818[b] ±0.406
C. punctatus	14.240[a] ±1.03	9.344[b] ±0.407	8.124[b] ±0.351	10.640[c] ±0.235

Means with different superscript in a row, differs significantly $P<0.01$.

exposed to 50 ppm of clove oil. In *C. punctatus,* significant ($P<0.01$) higher duration of 14.240 ±1.03 min was recorded for 50 ppm dose, while specimens exposed to 200 ppm dose of clove oil recovered in 10.640 ±0.235 min, which was significantly different from time duration required for 100 and 150 ppm treatment groups. The time required for inducing anesthesia with clove oil 200 ppm was lowest in both species under study. At this concentration the recovery period was highest in *C. gariepinus* and higher in *C. punctatus.* Hence, 200 ppm of clove oil can be recommended for anesthetizing these species.

Data are not available regarding the efficacy of clove oil as anesthetic in species selected for the present study, however,effect of clove oil was studied in other species and a dose 25 mg/liter was found effective in anesthetizing Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon, (*O. kitsutch*), rainbow trout, (*O. mykiss*) and white sturgeon (*Acipenser transmonatanus*) (Taylor 1999). Keene *et al.* (1998) observed that 40-60 ppm was effective in anesthetizing juveniles of rainbow trout *O. mykiss.* Munday and Wilson (1999) studied the comparative efficacy of clove oil and other anesthetic agents in coral reef fish (*Pomacentrus amboinensis*) and reported that clove oil was superior to benzocaine, MS-222 and 2-phenoxyethanol in inducing anesthesia, however, it was marginally less effective than quinaldine. Reports are also available regarding the cost comparison between clove oil and MS-222, for inducing anesthesia in fishes, which showed that clove oil, is much cheaper and safe anesthetics as compared to MS- 222 (Keene *et al.* 1998). Synthetic anesthetic agents like MS-222 are expensive and difficult to obtain in developing countries like India; further, they may also have potential harmful side effect on humans especially when the anesthetics are used to capture wild fishes meant for human consumption. Clove oil is both inexpensive and easily available in India and it is recognized as safe in humans. As found in the present investigation clove oil was effective even in very low concentration. Further the recovery time in fishes was also short. Thus all these features make clove oil a safe anesthetic agent, which can be advocated for anesthetization of fishes.

SUMMARY

The efficacy of clove oil as an anesthetic agent was studied in *Channa punctatus* and *Clarias gariepinus* by exposing to different concentrations of clove oil. The concentration of 200 ppm was ideal in inducing anesthesia as well as recovery from anesthesia.

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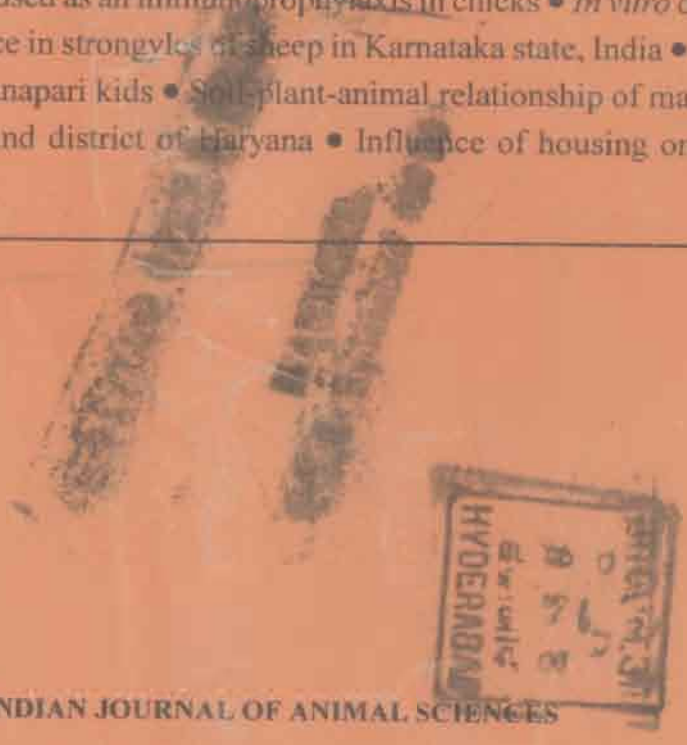

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